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Editorial assistant: Roselaine Monteiro
roselaine@endocrino.org.br

Rua Botucatu, 572 – conjunto 83 – 04023-062 – São Paulo, SP
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Segmento Farma editores

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Valuable insights from real-life experiences of advanced thyroid cancer treatment with sorafenib in Latin America

Evelin C. Farias[1]
https://orcid.org/0000-0002-9832-0269

Ana Oliveira Hoff[1]
https://orcid.org/0000-0002-7058-6321

[1] Instituto do Câncer do Estado de São Paulo (ICESP), Faculdade de Medicina da Universidade de São Paulo (FMUSP), São Paulo, SP, Brasil

Approximately 10,000 new cases of thyroid cancer are diagnosed annually in Brazil (1), most of the cases are associated with a survival rate of over 98% 5 years after diagnosis, but 10-20% present or eventually develop distant metastases (2,3). Until recently, the only effective treatment for distant metastatic disease was radioactive iodine (RAI) and the prognosis of patients who failed RAI therapy was poor, the 10-yr survival rate being only 10% (4). From these, patients with enlarging and FDG-PET positive lesions are the ones with the lowest rates of survival (5). With the knowledge that oncogenic kinases play a significant role in tumorigenesis and disease progression several kinase inhibitors have been investigated and became approved therapies. Based on phase III, placebo-controlled trials, multi-kinase inhibitors such as sorafenib (DECISION) and Lenvatinib (SELECT) have been approved by the Federal and Drug Administration (FDA) in 2013 and 2015 and by ANVISA in 2015 and 2016, respectively.

In the DECISION trial (N=417), patients on sorafenib had significantly longer progression-free survival (PFS) compared to placebo (10.8 months versus 5.8 months (HR: 0.59; IC 95%: 0.45 – 0.76; $p < 0.0001$) as well as a higher response rate (RR) (12.2% versus 0.5%) with no difference in overall survival (OS). The most common adverse events, observed in more than 50% of patients, included hand and foot skin syndrome (HFS), diarrhea, alopecia, and rash. Hypertension was observed in 40.6% of patients (6).

In the SELECT trial (N=392), the median PFS was 18.3 months in those who received lenvatinib compared to 3.6 months in the placebo group (HR 0.21; IC 99%: 0,14 – 0.31; $p < 0.001$) and the RR was 64.8% versus 1.5% in the placebo arm. Taking into consideration the entire cohort there was no significant improvement in overall survival, but subgroup analysis identified improved OS in older patients (> 65 years) and in patients with lung metastases (> 1 cm) (7-9). Adverse events were frequent, 97.3% of patients experienced some form of an adverse event. Most frequently, patients experienced hypertension (67.8%), diarrhea (59.4%), fatigue (59%), weight loss among others (7).

New therapies for radioactive iodine refractory advanced thyroid cancer continue to emerge. Precision medicine has become a reality, mainly in private medicine, and the treatment is switching from promiscuous multi-targeted kinase inhibitors to the specific inhibition of the mutated pathway found in tumor genotyping.

Correspondence to:
Evelin C. Farias
evelin_cf@hotmail.com

Ana O. Hoff
ana.hoff@hc.fm.usp.br

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These more specific inhibitors associate significantly stronger inhibition with fewer adverse events. In this context, NTRK inhibitors such as Larotrectinib have been approved by Federal Agencies in the US, Europe, and Brazil; BRAF and MEK inhibitors such as dabrafenib with trametinib have FDA approval for anaplastic thyroid cancer that harbors a *BRAF* V600E mutation. Selpercatinib and Praseltinib were recently approved for *RET*-mediated cancers including medullary thyroid cancer and differentiated thyroid cancers (10-13).

In this issue of the *Archives of Endocrinology and Metabolism*, real-life experiences from two independent cancer centers in Latin America are published. The study by Fierro-Maya and cols. from Colombia reports the safety and efficacy of sorafenib in 19 patients with advanced differentiated thyroid cancer (DTC). This was a prospective, phase II study that included patients with iodine-refractory and progressive DTC. The primary outcome was RR by RECIST 1.1 criteria. Secondary outcomes included PFS, OS, duration of response, and safety. Eligible patients were initiated on sorafenib 400 mg twice a day and were followed 1 month after initiation of therapy and at 3-month intervals. Patients were allowed to have dose reductions according to the severity of adverse events. From 19 patients enrolled in the study, 6 were excluded from efficacy analysis since they did not complete 1 month of therapy. As expected, more than 80% of patients had papillary thyroid cancer (PTC), most had good performance status (ECOG 0 or 1), but 14 patients (73%) had a history of unspecified cardiovascular morbidity. From 13 evaluable responses, 5 had a partial response (PR) (35.7%) with a mean duration of 10.8 months, 6 patients had stable disease (SD) and 3 had progression of disease (PD). Median PFS and OS were not reached at the planned 2-yr follow-up period but were estimated as 18 months (mean, 95% CI 10.7-20.3) and 21.3 months (mean, 95% CI 17.8-24.8), respectively. In terms of significant AEs, HFS was observed in 68% of patients, diarrhea in 57%, hypertension in roughly 100% of patients. In addition, one patient had a myocardial infarction, and another patient suffered a sudden death possibly from a ruptured aortic aneurysm (14).

The study by Treitsman and cols. is a retrospective review of 44 patients with advanced DTC refractory to radioactive iodine therapy treated with sorafenib (N=40) or vandetanib (N=4) upon documented disease progression. In addition to analysis of RR, PFS and AEs, the authors compared tumor and clinical characteristics between patients with distinct outcomes related to disease progression and death, in an attempt to identify prognostic factors associated with good response to therapy. At this Institution, the indication to start MKI therapy included disease progression with symptoms, or asymptomatic with extensive disease. Patients that were asymptomatic with lesions < 2 cm were not started on therapy despite progression. Patients were followed by a multidisciplinary team at 15 or 30-day intervals for dose adjustments and to manage side effects. Similar to other studies, PTC was the most frequent histology, and lung was the most frequent site of metastases (91%), with 20% of patients having only lung metastases. Regarding the response to therapy, the authors observed very favorable responses, 1 patient had a complete response (CR), 9 PR (20.4%), 22 SD (50%), and 12 PD (27.3%), with a median PFS of 24 months and median OS of 31 months. Of 44 patients, 43 developed an AE, most were grade 1 or 2 (83%); half required drug interruption, and adjustment and 25% discontinued the drug due to an adverse event. The most frequent AEs were HFS (68.2%), diarrhea (70.4%), and fatigue (70.4%); hypertension was observed in 11.3%. According to univariate analysis, having a target lesion with high SUV in PET-CT, larger primary tumor size, and several metastatic sites were associated with poor response while having lung-only metastases and lower thyroglobulin levels during therapy were associated with better outcome (15).

Both studies provide valuable insights regarding the treatment of advanced thyroid cancer in Latin America where most patients depend on the public health system with limited resources, with sorafenib being the primary or only available drug. First, they demonstrate very favorable responses to therapy with patients benefiting from symptomatic improvement and prolonged PFS. In both studies, PFS was longer than observed in phase III DECISION trial but similar to other real-life experiences published to date (14). One possible explanation for this is the fact that in clinical practice, physicians might use less stringent criteria to define disease progression during treatment, especially given continued clinical benefit and lack of other therapeutic options. In regard to better response rate, one possible reason could be the predominance of lung metastases in both studies. Another important piece of information obtained from the INCA study (1) is the fact that initiating therapy at a later timepoint (target

lesions > 2 cm) did not impact response rate and PFS. However, at the same time, it was clear that patients with more aggressive disease (higher SUV in PET-CT and extensive metastases) should not have initiation of treatment delayed. Despite these encouraging reports, one important message that should be drawn from both experiences is the rate of adverse events, therefore careful patient selection before initiation of therapy, better control of comorbidities (hypertension), and a close follow-up with frequent clinical visits performed by a multidisciplinary team is of utmost importance to avoid undesirable treatment discontinuation.

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Safety and efficacy of sorafenib in patients with advanced thyroid carcinoma: a phase II study (NCT02084732)

[1] Unidad de Endocrinología Oncológica, Instituto Nacional de Cancerología, Bogotá, Colombia
[2] Instituto Nacional de Cancerología, Endocrinóloga en Colsanitas, Bogotá, Colombia
[3] Servicio de Endocrinología, Hospital Universitario San Ignacio, Bogotá, Colombia
[4] Unidad de Investigaciones, Instituto Nacional de Cancerología, Bogotá, Colombia
[5] Unidad de Imágenes Diagnósticas, Instituto Nacional de Cancerología, Bogotá, Colombia

Luis Felipe Fierro-Maya[1]
https://orcid.org/0000-0003-1661-6574

Gloria Garavito González[1,2]
https://orcid.org/0000-0003-4820-3061

Leonardo Javier Rojas Melo[1,3]
https://orcid.org/0000-0002-8876-937X

Andrés Arturo Cuéllar Cuéllar[1]
https://orcid.org/0000-0002-1757-7952

Alexander Carreño[4]
https://orcid.org/0000-0003-3716-1564

Claudia Córdoba[5]
https://orcid.org/0000-0002-8940-6554

Correspondence to:
Luis Felipe Fierro-Maya
Calle 1, 9-85, Bogotá, Colombia
ffierro@cancer.gov.co

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ABSTRACT

Objective: Sorafenib significantly prolonged progression-free survival in patients with iodine-refractory advanced thyroid cancer. The present study was initiated before sorafenib was approved in Colombia and therefore represents an effort by an oncology institution to evaluate its efficacy and safety in this population. **Subjects and methods:** This phase II clinical trial had a single treatment arm. We included adult patients with progressive metastatic iodine-refractory thyroid cancer who received treatment with sorafenib 800 mg/day (400 mg every 12 hours) up to a maximum of 24 months or until the occurrence of limiting related toxicity, the progression of the disease, or voluntary withdrawal. **Results:** Nineteen patients received the treatment and were included in the safety analysis. However, for the efficacy analysis, 6 patients were excluded because they received only one month of therapy. Thirteen (68%) patients were women, and the mean age at diagnosis was 61.8 years. No complete responses were observed; 5 patients had a partial response (35.7%), 6 patients had stable disease, and 3 showed progression. Mean progression-free survival was calculated at 18 months (95% CI 10.7-20.3). Overall survival was estimated at 21.3 months (95% CI 17.8-24.8). **Conclusion:** For the first time in Colombia, the efficacy of sorafenib was evaluated in patients with advanced and progressive thyroid carcinoma refractory to radioactive iodine, with an efficacy and a safety profile similar to those previously reported. Arch Endocrinol Metab. 2021;65(4):404-10

Keywords
Thyroid carcinoma; metastasis; sorafenib tosylate

INTRODUCTION

Differentiated thyroid carcinoma (DTC) represents 90% of thyroid tumors, with 85% corresponding to papillar histology. Although the disease presents with a relatively indolent course, a significant percentage of patients develop locoregional relapses that can be managed by surgical resection or complementary radioactive iodine treatment. Overall survival (OS) in this setting is favorable, with 98% of patients still alive 10 years after diagnosis (1). However, approximately 7% to 23% of patients may develop distant metastases, of which up to two thirds may become refractory to radioactive iodine. The 10-year OS rate in this group is 40% to 42% (2). Patients with advanced disease who fail to respond to radioactive iodine require other therapeutic options. For this subset of individuals, and based on the knowledge of some of the genetic mutations within thyroid tumors (3), therapies inhibiting molecular targets such as tyrosine kinases have been developed. Sorafenib, a multikinase inhibitor with antiangiogenic action, was evaluated in the DECISION trial and was found to be effective in significantly prolonging progression-free survival (PFS) (4). These results, in turn, led to FDA approval for the treatment of advanced

thyroid cancer in November 2013 (5). Subsequently, the use of another multikinase inhibitor, lenvatinib, was also approved in February 2015 (6), based on the results of the SELECT study, which showed significant differences compared to placebo in terms of PFS tumor response rate (7). The present study was initiated before sorafenib was approved by the regulatory entity in Colombia (INVIMA) and therefore represents an effort by an oncology institution to evaluate its efficacy and safety in a group of patients with differentiated thyroid carcinoma considered advanced, inoperable, iodine-refractory, and with metastatic involvement in progression.

SUBJECTS AND METHODS

Study design

This phase II clinical trial had a single treatment arm with the main objective of evaluating the efficacy and safety profile of sorafenib during a 2-year follow-up period in patients with differentiated thyroid carcinoma with inoperable locally advanced or metastatic disease involvement, refractory to radioactive iodine, in progression. The primary outcome was objective response rate according to RECIST 1.1 criteria. Disease progression (PD) was defined as a 20% increase in the sum of target lesions. A partial response (PR) was considered a decrease of ≥30% in the sum of the longest diameters of the target lesions compared to the initial value. A complete response (CR) was defined as the disappearance of all target lesions, and the disease was considered stable when the criteria for PD or PR were not met. Additional outcomes included PFS, OS, response duration, frequency and distribution of medication-related adverse effects, and the quality of life of patients at each follow-up visit. These were assessed using the Functional Assessment of Cancer Therapy, General (FACT-G) scale. This outcome is under analysis and will be presented in a future publication.

Patients and treatments

The target population involved patients treated at the National Cancer Institute of Colombia (INC-ESE) diagnosed with advanced thyroid cancer who received treatment with sorafenib 800 mg/day (400 mg every 12 hours) up to a maximum of 24 months or until the occurrence of limiting toxicity, demonstrated disease progression, or voluntary withdrawal. All patients provided written informed consent before enrollment. Accrual was done sequentially after verification of inclusion criteria. These were as follows: adult patients >18 years old with confirmed histological diagnosis of differentiated thyroid cancer with inoperable locoregional or metastatic iodine-refractory involvement, which was defined as (8) a target lesion that does not show uptake in a gammagraphic evaluation after a given therapeutic dose under adequate conditions of elevated TSH and a low iodine diet; lesions that, despite showing iodine uptake, show progression after a dose of 100 mCi given in the previous 16 months; and patients showing progression after a cumulative dose greater than 600 mCi. All participants must have had tumor progression in the 12 months before enrollment, as suggested by several reports (9,10) and confirmed by the Response Evaluation Criteria in Solid Tumors, (RECIST version 1.1) (11), exhibit adequate functional status based on the Eastern Cooperative Oncology Group (ECOG) performance status (ECOG ≤ 2), and have a life expectancy of greater than 3 months. Previous use of chemotherapy, immunotherapy, or radiotherapy on the target lesion was permitted if the last dose was administered 4 weeks before study entry. Patients with uncontrolled cardiovascular diseases, unhealed wounds or ulcers, history of major bleeding in the 12 weeks prior to recruitment, thrombotic events in the last 6 months, or major surgery in the previous 4 weeks were excluded.

Follow-up

Patients were evaluated at baseline, after the first month, and then quarterly with physical examinations, electrocardiograms, and laboratory tests. Imaginological evaluations were performed using chest tomography and neck ultrasound at Months 0, 3, 6, 12, 18, and 24 to evaluate treatment response (RECIST version 1.1 solid tumor response criteria (11)). To evaluate toxicity, the classification system used in version 4.0 of the Common Terminology Criteria for Adverse Events (CTCAE) was used (12). Based on previous studies and the manufacturer's brochure, 4 levels of sorafenib dose reduction were used according to the severity of adverse events (Level 0: 800 mg per day, Level 1: 600 mg per day, Level 2: 400 mg per day, and Level 3: 200 mg per day).

Statistical analysis

For statistical analysis, descriptive parametric and non-parametric statistics were included as appropriate. In

the case of the qualitative variables, McNemar's test was used. For quantitative variables, paired Student's t-test and Wilcoxon Rank Sum tests were used for normally and non-normally distributed data, respectively. The Kaplan-Meier method was chosen to describe PFS and OS. Correlation analyses were performed between some markers and clinical variables and the observed outcomes using parametric and non-parametric coefficients.

This study was approved by the institutional research and ethics committee and was registered at Clinicaltrials.gov with the number NCT02084732 on March 12, 2014. Clinical monitoring as defined by the institutional research guidelines was conducted.

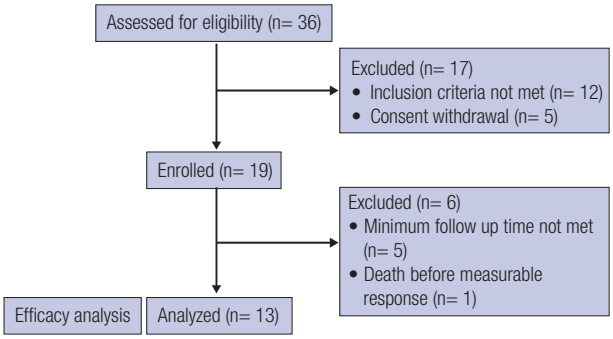

Figure 1. Patient flow diagram according to CONSORT guidelines.

RESULTS

Between August 2014 and November 2016, 36 patients were screened. Of these, 12 were not eligible, and 5 withdrew their consent. In the end, 19 patients were included in the study. However, for the efficacy analysis, 6 patients were excluded because they received only one month of therapy. Figure 1 presents patient flow according to the CONSORT guidelines. Thirteen (68%) patients were women, and the mean age at diagnosis was 61.8 years (range 38-84). All patients had metastatic lung involvement, 3 patients (15.7%) had additional bone involvement, and 10 patients (52.6%) had inoperable cervical metastases. All patients were subjected to locoregional resection at some time during the disease course. Baseline characteristics and prior treatments are summarized in Table 1. Time from diagnosis to inclusion in the study was calculated with a median of 7.1 years (range 1.8-25.4).

Median treatment time was 9.5 months (range 0.9-24.7). Twelve patients (63.1%) received sorafenib for more than 6 months, and 9 patients (47.3%) received it for at least 12 months. Tumor response could be evaluated in 13 patients. No complete responses were observed, but 5 patients experienced a partial response (35.7%) with a mean duration of 10.8 months on average. Furthermore, 6 patients had stable disease, and 3 showed progression. The best observed tumor responses are presented in Figure 2.

Concerning PFS and OS, median values were not achieved for the cohort. An estimated mean PFS was calculated at 18 months (95% CI 10.7-20.3), whereas mean OS reached 21.3 months (95% CI 17.8-24.8). Survival curves are presented in Figures 3 and 4, respectively.

Table 1. Baseline Clinical characteristics of the included patients

Variable	Disaggregation	Number	Percentage
Sex	Male	6	31.6
	Female	13	68.4
ECOG*	0	13	68.4
	1	5	23.3
	2	1	5.3
Comorbidities	Cardiovascular	14	73.7
	Articular	2	10.5
	Others	3	15.8
Thyroid cancer subtype	Papillary	16	84.2
	Folicullar	2	10.5
	Hürthle cell	1	5.3
Non operable cervical compromise	Yes	10	52.3
	No	9	47.7
Local radio-therapy	Yes	4	21.1
	No	15	78.9
I-131 doses (Cumulate)	Median (rank)	419	(160-950)

* Eastern Cooperative Oncology Group (ECOG) performance status.

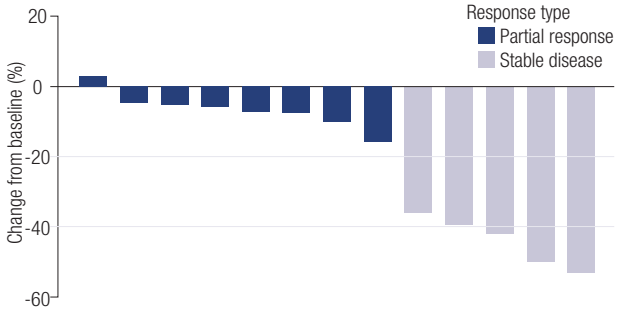

Figure 2. Best tumor response, RECIST criteria.

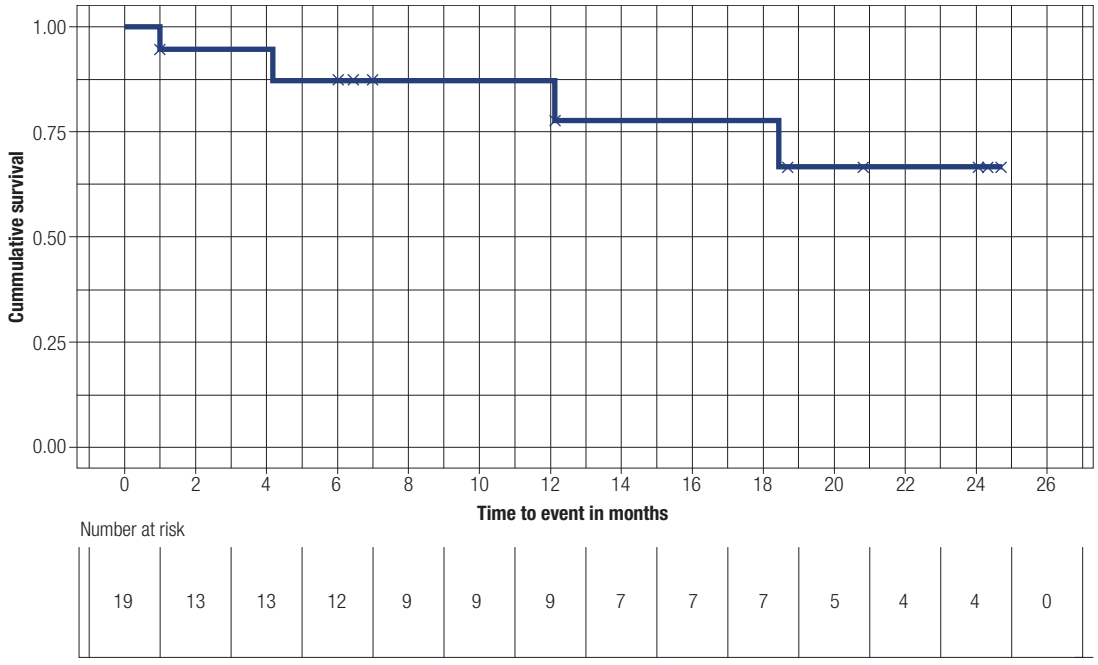

Figure 3. Progression free survival.
Total events: 3; Mean progression-free survival: 18 months (95% CI 10.7-20.3).

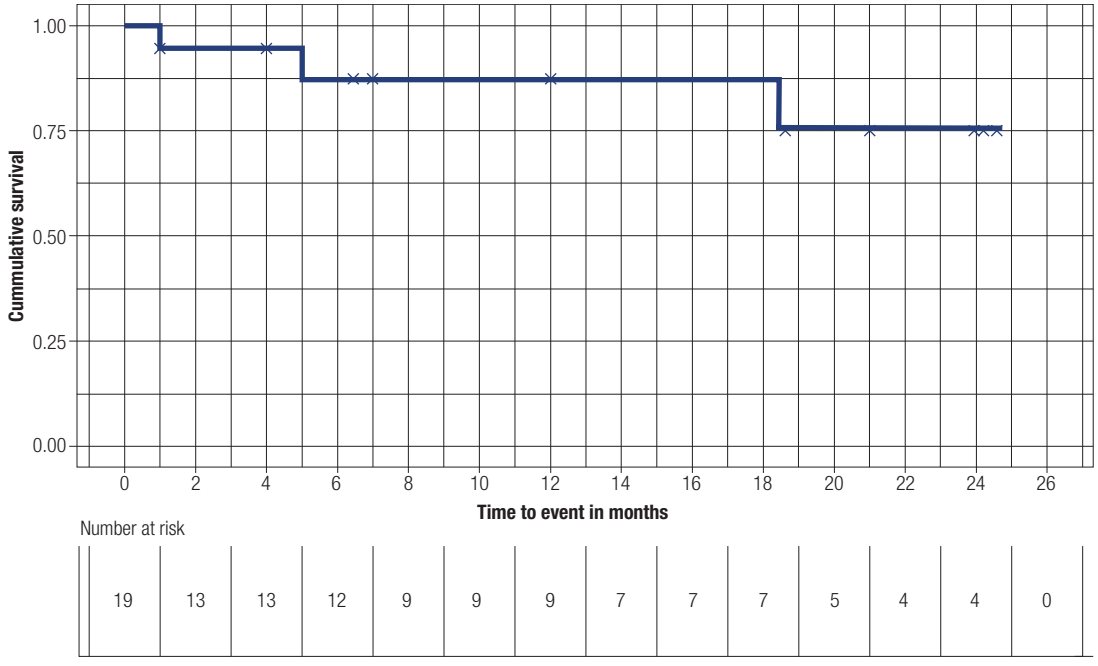

Figure 4. Overall survival.
Mean overall survival estimated at 21.3 months (95% CI 17.8-24.8).

With regard to thyroglobulin levels, no statistically significant differences (p = 0.075) were found between median values prior to treatment (1,223 ng/mL 95% CI 461-985) and the lowest achieved in the follow-up of patients receiving sorafenib (934 ng/mL 95% CI 473-1,394).

The most frequent adverse event was arterial hypertension, which occurred in all patients, followed by palmar-plantar erythema, which occurred in 13 of 19 patients (68.4%), and diarrhea, which occurred in 11 patients (57.8%). No deaths attributable to medication toxicity were documented. Table 2 summarizes adverse

events by frequency and severity. Dose reductions and interruptions due to adverse events occurred in 4 (21.0%) and 7 (36.8%) patients, respectively. Palmar-plantar erythema and arterial hypertension were the most common reasons for sorafenib interruption. Dose withdrawals occurred in 3 (15.7%) patients, all of them between Days 15 and 21 of treatment. One experienced a Grade 3 skin rash, and the other 2 experienced Grade 3 hand-foot erythema. Despite resolution of symptoms, these patients withdrew consent to participate in the study. Grade 3 adverse events occurred in 8 (42.1%) patients, mainly hypertension and hand-foot erythema.

A total of 3 patients (15.7%) died during the study period. The first patient experienced severe abdominal pain (10/10) after 20 days of treatment. Following this event, sudden death was declared. Due to non-consent on the part of the family to conduct an autopsy, specific organ alteration could not be confirmed. With suspicion of a ruptured abdominal aortic aneurysm, this event was reported to the regulatory agency overseeing the trial. Because no specific cause of death could be confirmed, it was not included in Table 2. Another patient had a serious adverse event (myocardial infarction) at 6 months of treatment; medication was discontinued, and the patient subsequently died of tumor progression 3 months after withdrawal. The final patient presented with disease progression under treatment at 18 months and died after one month of sorafenib withdrawal.

DISCUSSION

The frequency of distant metastases at diagnosis in differentiated thyroid carcinoma varies according to the histological type. It is estimated at approximately 10% in papillary carcinomas, 25% in follicular carcinoma, and up to 35% in Hürtle cell carcinoma (13-15). Pulmonary metastatic involvement is the most frequently involved site and was present in 100% of the patients in the current study. In addition, the presence of coexisting regional lymph node involvement should be kept in mind, given the morbidity that it entails. In the present study's population, 52% of the patients had inoperable cervical involvement, a figure that contrasts with the presence of local involvement in the DECISION trial of only 3.4% (4).

The 10-year survival prognosis in patients with metastases varies according to the age at the time of diagnosis; it is 95% in patients younger than 45 years, but it is reduced to 50% in patients older than 45 years (14,15). In this study, the proportion of reported deaths (15.7%) was greater than the 5% of deaths reported in the DECISION Trial (4), and the 3.3%

Table 2. Adverse events

Type	Description	Grade 1-2 n (%)	Grade 3 n (%)	Grade 4 n (%)
Dermatologic	Rash	4 (21.0)	3 (15.7)	0
	Stomatitis	4 (21.0)	1 (5.2)	0
	Hand/food Erythema	7 (36.8)	6 (31.5)	0
	Alopecia	1 (5.2)	0	0
General symptoms	Muscle pain	4 (21.0)	1 (5.2)	0
	Fever	1 (5.2)	0	0
	Weight loss	3 (15.7)	0	0
Gastro-intestinal	Diarrhea	6 (31.5)	4 (21.0)	1 (5.2)
	Nausea/vomiting	3 (15.7)	1 (5.2)	0
	Dysphagia	1 (5.2)	0	0
	ALT/AST elevation	1 (5.2)	0	0
	Cholangitis (biliary tract infection)	0	1 (5.2)	0
Pulmonary	Dyspnea/cough	0	1 (5.2)	0
Cardiovascular	Arterial hypertension	8 (42.1)	7 (36.8)	4 (21.0)
	Tachycardia	1 (5.2)	0	0
	Acute myocardial infarction	0	1 (5.2)	0
Other	Creatinine elevation	0	1 (5.2)	0

reported by Gupta-Abramson and cols. (16) but less than the 42% of deaths reported by Kloos and cols. (17). Bearing in mind that disease progression was the major cause of death, is important to mention that in the DECISION trial, 20.3% of patients randomized to sorafenib received systematic treatments after disease progression, unlike in this study, mainly because of the absence of the availability of other therapies. Another difference between the population included in both studies was the median time from diagnosis to inclusion, which was slightly longer in the present study (84 months, range 21-300) than in the DECISION trial (66 months, range 3.9-362).

The partial response (PR) rate was higher (35.7%) compared to observed in previously published phase II studies by Gupta-Abramson and cols. (16) and Kloos and cols. (17), which had PR rates of 28 and 15% respectively. It was also higher than the DECISION trial's PR rate of 12%. In the same way, the median PFS observed in our study (18 months) was longer than in the DECISION TRIAL (10.4 months) but similar to the PFS found by Gupta-Abramson and cols. (19.7 months) and by Kloos and cols. (16 months).

It corroborates the benefit of sorafenib in patients in whom the disease progresses and who lack other therapeutic options. Considering the very small percentage of Hispanic patients included in the DECISION trial (~1%), without being able to compute statistical comparisons, it is possible that tumor behavior could have differed in our population, thus warranting our conducting this phase II study in local and Hispanic populations. Furthermore, considering the benefit of prolonged median PFS for patients harboring somatic BRAF V600E mutations compared to their BRAF wild type counterparts (20.5 versus 8.9 months respectively), it is safe to assume that populations with higher mutational incidence can benefit from treatment with sorafenib to a greater extent. In addition, the prevalence of BRAF V600E mutations in DTC patients from Colombia has been reported to be between 60% and 66%, which is higher than reported in the DECISION trial (30%) (18,19). This might explain the high response rates observed in this study.

The most frequent serious side effects in this study were different than those reported in other studies (4,16,17) and involved the cardiovascular system, with arterial hypertension in 4 patients (21%) and acute myocardial infarction in 1 (5.2%). It is important to mention that 14 (73.7%) patients in this study had cardiovascular comorbidities. In addition, hypertension as an adverse event of antiangiogenic agents can be a marker of effective target inhibition, a greater response rate, PFS, or even OS (20), potentially explaining these best-observed responses.

Although there is increasing evidence of the benefit of Lenvatinib use, its safety profile differs, probably due to a greater antiangiogenic effect. Moreover, there are some rare but potentially fatal adverse events reported with its use, such as tracheoesophageal fistula, gastrointestinal perforation, and reversible rear leukoencephalopathy syndrome (21). It is therefore pertinent to consider sorafenib as an alternative therapy in cases of possible contraindications, such as patients with proteinuria or high risk of serious adverse events (22,23).

As more has become known about the limitations of radioactive iodine in differentiated thyroid carcinoma (24), the use of tyrosine kinase inhibitors has increased for patients with advanced disease in progression, who would have been considered intractable before these molecules were available.

In conclusion, the present study demonstrates the benefit of sorafenib for palliative use in Colombian patients with advanced progressive DTC who lack any other therapeutic options. Results in the setting of advanced thyroid carcinoma refractory to radioactive iodine are comparable in efficacy and safety, similarly to previous conclusions of other studies.

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Prognostic factors in patients with advanced differentiated thyroid cancer treated with multikinase inhibitors – a single Brazilian center experience

Natalia Treistman[1,2]
https://orcid.org/0000-0002-8084-5040

Gabriela Maia Nobre[1]
https://orcid.org/0000-0001-8362-1387

Mariana Yoshii Tramontin[1]
https://orcid.org/0000-0002-9168-414X

Gabriel Madeira Werberich da Silva[3]
https://orcid.org/0000-0003-0451-3017

Daniel Herchenhorn[2,4]
https://orcid.org/0000-0001-5166-848X

Luiz Henrique de Lima Araujo[3]
https://orcid.org/0000-0001-9486-7139

Fernanda Accioly de Andrade[1]
https://orcid.org/0000-0002-6687-0506

Rossana Corbo[1]
https://orcid.org/0000-0001-8725-7455

Daniel Bulzico[1]
https://orcid.org/0000-0003-1270-7241

Fernanda Vaisman[1,2]
https://orcid.org/0000-0002-6835-7108

[1] Departamento de Medicina, Serviço de Endocrinologia, Instituto Nacional do Câncer (Inca), Rio de Janeiro, RJ, Brasil
[2] Departamento de Medicina, Serviço de Endocrinologia, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil
[3] Departamento de Medicina, Serviço de Oncologia, Instituto Nacional do Câncer (Inca), Rio de Janeiro, RJ, Brasil
[4] Grupo de Oncologia D'Or, Instituto D'Or de Pesquisa e Educação (IDOR), Rio de Janeiro, RJ, Brasil

ABSTRACT

Objective: The aim of this study was to describe the real-world experience multikinase inhibitors (MKI) in the treatment advanced differentiated thyroid carcinoma (DTC) refractory to radioactive iodine (RAIR) therapy. **Subjects and methods:** We reviewed the records of all patients with MKI-treated DTC from 2010 to 2018. Progression free survival (PFS), response rates (RR) and adverse events (AE) profiles were assessed. Clinical parameters were compared between groups with different outcomes (disease progression and death) to identify possible prognostic factors and benefit from treatment. **Results:** Forty-four patients received MKI for progressive RAIR DTC. Median PFS was 24 months (10.2-37.7) and median overall survival (OS) was 31 months. Best overall response was complete response in one patient (4.5%), partial response in nine (20.4%), stable disease in twenty-two (50%), and progressive disease (PD) in twelve (27.3%). Seventy-two point 7 percent patients had clinical benefit and AE were mild in most cases (82.7%). Progressive patients were more likely to have FDG positive target lesion than those who did not progress (p = 0.033) and higher maximum SUV on target lesions (p = 0.042). Presence of lung-only metastasis and lower thyroglobulin (Tg) during treatment was associated with stable disease (p = 0.015 and 0,049, respectively). Patients with shorter survival had larger primary tumor size (p = 0.015) and higher maximum SUV on target lesions (p = 0.023). **Conclusion:** Our findings demonstrate safety and effectiveness of MKI in patients with advanced RAIR DTC. We were able to identify as possible prognostic markers of better outcomes: absence of FDG uptake on target lesions, lower maximum SUV on PET-CT, presence of lung-only metastasis and lower Tg during treatment. Arch Endocrinol Metab. 2021;65(4):411-20

Keywords

Differentiated thyroid cancer; radioactive iodine refractory; multikinase inhibitor therapy; real-world data

Correspondence to:
Fernanda Vaisman
Av. Padre Leonel Franca, 110, sala 505, Gávea – 22451-000 – Rio de Janeiro, RJ Brasil
fevaisman@globo.com

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INTRODUCTION

Differentiated thyroid carcinoma (DTC) is the most common endocrine malignancy and its incidence has been rising worldwide (1). In Brazil, estimates for 2018-2019 indicate 9610 new cases (2).

In general, DTC has excellent prognosis and over 98% 5-year overall survival (OS) rates. Despite representing about 3% of new cancer cases in the US, it is responsible for less than 0.3% of cancer-related deaths (3). However, there is a small group of patients that can have a worse prognosis and need for additional therapy besides surgery and radioactive iodine (RAI). It is also known that patients with metastatic disease sensitive to RAI have better outcome than those who are not (4).

For patients with advanced and metastatic disease who are refractory to RAI (RAIR), therapeutic options are limited and overall response rates (RR) are also modest. Historically, it is known that DTC has poor response to cytotoxic chemotherapy (5,6).

Over the last 15 years, knowledge on molecular mechanisms involved in DTC carcinogenesis and progression has evolved substantially, and with that new therapeutic possibilities were discovered (7-9). Multikinase inhibitors (MKI) were first used to treat hematologic malignancies, liver and renal cancers and were more recently approved for progressive RAIR DTC. In Brazil, the two approved MKI for RAIR DTC are sorafenib and lenvatinib, but those agents are not widely available for the public health system (10).

The experience of MKIs in DTC is still growing in many settings. Since the release of prospective controlled studies, many authors have published their experience with these agents in real-life scenarios and reported important differences in this context (11-31). However, Brazilian experience is still limited and there is no large DTC experience reported.

The aims of this study were to analyze and describe the experience of a Brazilian referral center in oncology with the use of MKI in the treatment of patients with advanced RAIR DTC and to identify predictive and prognostic factors associated with treatment.

SUBJECTS AND METHODS

We retrospectively reviewed medical records of all MKI-treated DTC patients at a single center – National Cancer Institute (Inca) –, Rio de Janeiro, Brazil, from December 2010 to November 2018.

Inclusion criteria were patients > 18 years diagnosed with advanced DTC treated with MKI. For our analysis, we included all patients, even those with short-term treatments (less than 3 months before progression, treatment discontinuation or death).

Patients younger than 18 years old, medullary thyroid carcinoma or anaplastic thyroid carcinoma, or patients with DTC not treated with MKI were excluded.

The following demographic and clinical data from all subjects included in the analysis were collected: gender, age at diagnosis, tumor histology, number of RAI treatments, cumulative RAI activity, whole body survey (WBS) results after therapeutic RAI, criteria used to determine RAIR disease, tumor staging, metastatic lesion sites, target lesion size and site, other systemic or localized therapies performed, adequate TSH suppression prior to MKI, date and dosage of MKI treatment initiation, dosage modification when it occurred, temporary discontinuation of treatment, adverse events (AE) and its degree when present, treatment discontinuation date and motive, anti-thyroglobulin (ATg) antibody levels and serum thyroglobulin (Tg) before treatment, lower ATg and Tg during treatment, imaging studies during follow-up and structural response, time of last visit during follow-up, date of death. Tumor stage was classified according to AJCC/TNM 8th edition (32).

Criteria used to determine RAIR disease was defined using the American Thyroid Association guidelines' definition (6,33).

Patients who had clinical and radiological progressive RAIR disease were evaluated for MKI therapy. Therapy was initiated in those with symptomatic progression or with disseminated disease not manageable with localized therapy. In general, therapy was not indicated in asymptomatic patients with target lesions smaller than 2 cm in the largest diameter. To be eligible for treatment, patients must have had documented disease progression within 14 months.

At our institution, patients on MKI therapy are followed by a multidisciplinary team, including endocrinology, oncology, dermatology, and nurses. Depending on the case, voice therapist, clinical pain specialist and others may be involved. Initial treatment with MKI requires shorter clinical reevaluations (every 15 to 30 days) for dose adjustments and management of possible AE, and then clinical and laboratory reassessment is performed every 2 to 3 months. The severity of AE is graded according to the National

Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Imaging and structural response studies were evaluated according to a certified radiologist (PD being defined as at least 20% increase in measurements and partial response [PR] as decrease in at least 30% of target lesions).

PFS was defined as the time between initiation of MKI therapy and the first documentation of radiological disease progression, death or loss of follow-up. OS was defined as the time between MKI therapy initiation and death, loss of follow-up or last clinical visit.

Functional sensitivity of the serum Tg assay varied over the years. From 2001 to 2010, serum Tg was quantified by immunometric assay (Immulite) with functional sensitivity of 0.2 ng/mL, and from 2010 to the present functional sensitivity dropped to 0.1 ng/mL (Elecsys Tg II test).

Dosages of ATg, TSH and free T4 are currently performed with electrochemiluminescence immunoassays. Functional sensitivity of ATg assay is currently 10.0 IU/mL (Elecsys Anti-Tg test). TSH Functional sensitivity is 0.005 μIU/mL (Elecsys TSH test) and free T4 is 0.5 pmol/L (Elecsys FT4 II test).

Ethical guidelines

This work has been approved by Inca's ethics research committee under the number 40788815.0.1001.0065.

Statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were described as means and medians, categorical variables, presented as numbers and percentages. Parametric variables were evaluated with chi-square and Student's t test. Nonparametric variables were evaluated by the Mann-Whitney U test. Survival curves were performed by the Kaplan-Meier method, and the log-rank test was used to determine statistical significance. The confidence interval is 95% and p value was considered statistically significant < 0.05.

RESULTS

In total, 44 patients were included in the analysis and their medical records were reviewed. Baseline characteristics are described in Table 1.

Twenty-seven (61.4%) patients were female and 17 (38.6%) male. Mean age at diagnosis was 60.8 and mean age at the beginning of MKI treatment 69.3 years. Regarding tumor histology, 31 patients (70.5%) had papillary thyroid carcinoma (PTC), 12 (27.2%) had follicular thyroid carcinoma (FTC) and 1 patient (2.3%) had poorly differentiated thyroid carcinoma (PDTC). Among PTC patients, 6 had follicular variant papillary, 3 patients had insular variant, 1 tall cell (13.6%, 6.8% and 2.3%, respectively). Regarding FTC, 3 patients had oncotic variant (6.8%).

Table 1. Baseline characteristics

	N = 44	%
Age (years)	60.8 (34-79)	
Sex F:M	27:17	61.4: 38.6
Size (cm)	4.6 (1.1-11.5)	-
Histology		
Papillary	31	70.5
Follicular	12	27.2
Poorly differentiated	1	2.3
Follicular variant papillary	6	13.6
Hürthle Cell	3	6.8
Insular	3	6.8
Tall cell	1	2.3
8th edition AJCC		
Tx	21	47.8
T1a	0	0
T1b	0	0
T2	6	13.6
T3a	4	9.0
T3b	1	2.3
T4a	7	15.9
T4b	5	11.4
Nx	31	70.5
N0	1	2.3
N1a	4	9.0
N1b	8	18.2
M1	23	52.2
At least one RAI treatment	41	93.2
RAI activity (mCi)	422.5 (150-1000)	-
Symptoms before MKI	20	45.5
Time from diagnosis to MKI (years)	68.7 (0.3-210.1)	-
Additional therapy besides MKI		
External beam radiation	27	61.4
Chemotherapy	3	6.8
Embolization	3	6.8
Zoledronate	6	13.6
Final status		
Stable disease	11	25
Complete response	1	2.3
Progression	7	15.9
Disease related death	25	56.8
PFS on MKI (months)	24 (10.2-37.7)	-
OS after MKI (months)	31 (17.7-44-.2)	-
Follow up (months)	99.6 (12.5-236.3)	-

MKI: multikinase inhibitors; PFS: progression free survival; OS: overall survival; RAI: radioiodine.

Twenty-three patients (52.2%) already had distant metastases at diagnosis. Forty-one patients were treated with RAI. They received median cumulative activity of 422.5 mCi (150-1,000). Three patients were not treated with RAI due to unresectable disease and large remaining volume of thyroid tissue.

Criteria used to determine RAIR disease was negative WBS in 40.5%, PD less than 16 months after RAI treatment in 27.5% and cumulative RAI activity over 600mCi without remission of disease in 27.5% of cases.

Regarding metastatic lesions sites, 40 patients (91%) had pulmonary metastasis, 9 of those (20.45%) had exclusively pulmonary metastasis. Sixteen patients (36.3%) had bone metastasis and 10 (22.7%) patients had metastasis in other sites, including liver, pancreas and the pituitary gland. Target lesions were pulmonary in 27 cases (61.4%), cervical masses or lymph nodes in 8 cases (18.2%), bone metastasis in 5 cases (11.4%) and 4 cases had target lesions located in other areas. Average target lesions size was 3.1 cm. All patients were evaluated with PET-CT, except one.

Median time between DTC diagnosis and initiation of MKI therapy was 68.7 months (0.3-210.1). Forty patients used sorafenib and 4 patients used vandetanib, all as first line treatment. Average initial dose of sorafenib was 760 mg/d, with 36 patients starting 800 mg. Initial dose for vandetanib varied between 100 mg/d and 300 mg/d.

Prior to MKI therapy 45.5% of patients had adequate TSH suppression (TSH < 0.1 μIU/mL at least 9 of 12 previous months). Mean serum Tg before MKI was 6,469.4 ng/mL and mean ATg titers 197.4 IU/mL, mean lowest Tg during MKI treatment and lowest ATg during MKI treatment were 804.3 ng/mL and 57.9 IU/mL, respectively.

Regarding best response during treatment with MKI, 9 (20.4%) patients had PR, 22 patients (50%) had SD and 12 cases (27.3%) had PD as best response during treatment as shown in Table 2. One patient presented complete response (CR) criteria and this case will be further discussed later. Overall, 72.7% patients had clinical benefit from MKI treatment, defined as the sum of CR, PR and SD. Twenty patients had symptomatic disease before starting MKI. 13 of them (65%) reported clinical improvement of symptoms some time during treatment.

Median PFS was 24 months (10.2-37.7) (Figure 1) and median OS was 31 months (17.7-44.2) (Figure 2). Median follow-up of 99.6 (12.5-236.3) months. Duration of response for the entire cohort was 12 months (0.5-800, for PR 12 months (8-35), for SD 31 (5-80) and for PD 9 (0-31 months).

Forty-three patients presented AE during treatment, only 1 patient had no AE reported (results in Table 3). In total, 168 AE were described, 139 (82.7%) mild

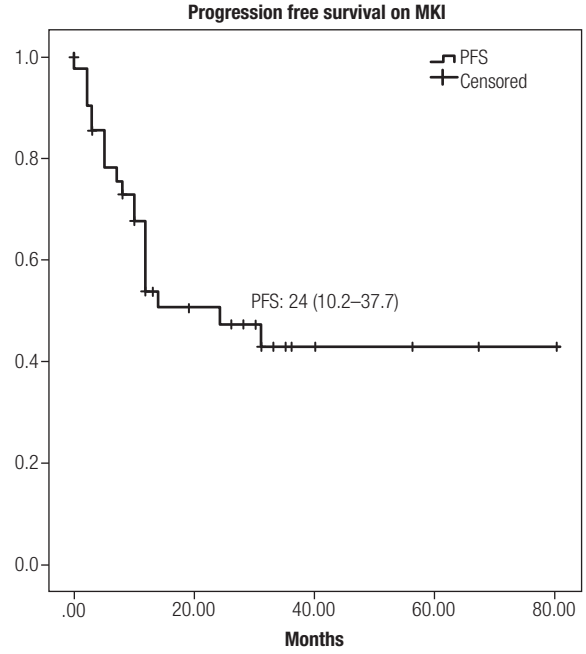

Figure 1. Progression free survival during MKI (in months).

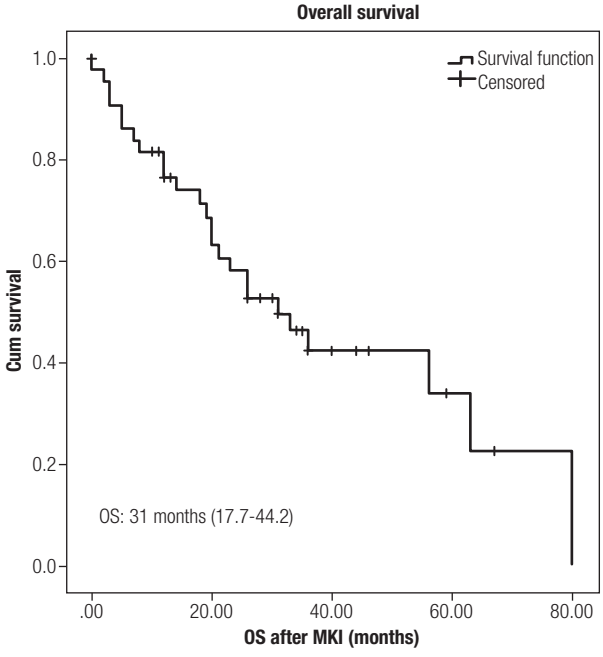

Figure 2. Overall survival after MKI (in months).

(grades 1 or 2) and 29 (17.3%) grade 3 or 4. 21 patients (47.7%) required temporary discontinuation of medication due to AE. Twenty-two patients (50%) required dose reduction and 11 cases (25%) had the drug suspended due to AE. One patient had cutaneous neoplasia secondary to MKI use.

Table 2. Response to therapy RECIST 1.1

N = 44	Best response to MKI therapy
Complete response	1 (2.3%)
Partial response	9 (20.4%)
Stable disease	22 (50%)
Clinical benefit	32 (72.7%)
Disease progression	12 (27.3%)

MKI: multikinase inhibitors.

We also analyzed and compared data from patients who had PD on MKI with those who did not have PD while using MKI. These analyses are presented in Table 4. We found no difference between the group that progressed and those that did not progress regarding age, gender, symptoms at the beginning of MKI treatment, number of metastatic sites, number of AE or average RAI activity.

On uni-variate analysis patients who had PD on MKI were more likely to have FDG uptake on target lesions on PET-CT when compared to patients who did not progress (p = 0.033) and higher maximum SUV on PET-CT on target lesions (p = 0.042). Presence of lung-only metastasis was associated with no PD (p=0.021). Patients who did not progress had on average lower Tg during treatment when compared to

Table 3. Adverse events during MKI treatment

	Any grade	G1-G2 (%)	G3-G4 (%)
Hand-foot syndrome	30	22 (50)	8 (18.2)
Diarrhea	31	25 (56.8)	6 (13.6)
Fatigue	31	29 (65.9)	2 (4.6)
Hypertension	5	4 (9.0)	1 (2.3)
Alopecia	11	9 (20.4)	3 (6.8)
Anorexia	5	5 (11.3)	0
Weight loss	8	8 (18.2)	0
Nausea	9	8 (18.2)	1 (2.3)
Rash	7	5 (11.3)	2 (4.6)
Hematologic toxicity	1	1 (2.3)	0
Pruritus	1	1 (2.3)	0
Secondary neoplasia	1	0	1 (2.3)

MKI: multikinase inhibitors.

Table 4. Progression on MKI

	Progression (22)	No progression (22)	p-value
Age (years)	59	66	0.561
Sex (F)	50%	72.7%	0.215
Primary tumor size (cm)	4.5	3.7	0.057
Number of metastatic sites			0.03
1	13.6%	45.5%	
2	45.5%	31,8%	
3	22.7%	18.2%	
4	18.2%	4.5%	
Pulmonary metastasis only	4.5%	36.36%	**0.02**
Max. SUV - PET + Target lesion	14.62	11.0	**0.042**
PET + Target lesion	100%	85.7%	**0.033**
Lowest Tg during MKI	664.9	165.5	**0.049**
Symptomatic disease	54.5%	36.4%	0.364
Number of AE	3.0	4.0	0.213
Mean RAI activity	365.79	473.81	0.057

MKI: multikinase inhibitors; Tg: thyroglobulin; RAI: radioiodine, AE: adverse events.

patients who progressed (p = 0.049), however there was no statistically significant correlation with initial Tg.

We performed analysis comparing patients who died during or after MKI treatment and survivors, as shown in Table 5.

Patients who died had larger primary tumor size (p = 0.035), more frequently had more than one site of distant metastasis (p = 0.002) and higher incidence of glucose uptake on target lesions on PET-CT (p = 0.023).

DISCUSSION

In this study we describe a retrospective cohort of patients with progressive unresectable DTC RAIR, treated with MKI for a median period of 99.6 months in a public referral center in Rio de Janeiro. This larger Brazilian experience showed that, in a real-world study, median PFS was 24 months (10.2-37.7) and OS was 31 months (17.7-44.2), with frequent but manageable adverse events in properly selected patients.

Despite the favorable results of previous phase III studies, there are still many unresolved questions regarding the clinical management of patients treated with MKI treated RAIR DTC. Chief among them is how such results are converted to a real-life scenario practice. Several groups have begun to describe their experience with treating DTC using MKI and its feasibility in many different countries, continents, and contexts (13-31,34,35). Findings of previous colleagues as well as our results are summarized in Table 6. Our study represents a large single center cohort treated with MKI, with long follow-up, being one of the few cohorts in South American and the first with Brazilian population.

Regarding survival outcomes, our findings are slightly different from previous phase III studies but consistent with other groups reports of real-world experience, such as Cabanillas and cols. with 19 months PFS in a North American cohort, Benekli and cols. with 21.3 months PFS in Turkish population, Molina-Vega and cols. with 18 months PFS in a Spanish cohort, Sugino and cols. 24.3 months in a Japanese cohort, and Jerkovich and cols. with 31.5 months PFS in an Argentinian cohort (11-13,18,25,26,29).

Clinical trials DECISION and SELECT have previously showed PFS of 10.8 months and 18.3 months, respectively, an improvement when compared to their placebo groups, respectively, 5.8 and 3.6 months (11,12). Although not directly comparable, considering all our subjects presented documented PD within 14 months prior to MKI initiation, we believe our finding of median 24 months PFS demonstrates the usefulness of MKI treatment to prevent disease progression.

Most of our patients experienced clinical benefit of treatment. 50% of them had SD, 20.4% PR and one presented CR. This patient was started on MKI after presenting a rapidly progressive unresectable endotracheal lesion that can no longer be seen on cross sectional images after 28 months of sorafenib. Our 72.7% clinical benefit was similar to Marotta and cols.

Table 5. Disease related death

	Deaths (25)	Survivors (19)	p-value
Age	61.16	60.42	0.649
Sex (F)	72%	47.4%	0.125
Primary tumor size	5.47	3.6	**0.035**
Number of metastatic sites			
1	20%	42.2%	**0.02**
2	44%	31.5%	
3	16%	26.3%	
4	20%	0%	
PET + target lesion	96%	84.2%	**0.023**
Lowest Tg during MKI	914.11	701.75	0.088
Symptomatic disease	56%	31.6%	0.135
PD target lesion vs. Non target lesion	64.7%	75%	0.689
Number of AE	3.6	4.1	0.530
Mean RAI activity	452.27	386.11	0.407

MKI: multikinase inhibitors; Tg: thyroglobulin; RAI: radioiodine, AE: adverse events.

Table 6. Review of world real-life experience in use of MKI in DTC

Country	Year	Authors	Number of centers	Drugs	Number of subjects	1st line MKI or more	Median PFS (months)	Prognostic Factors
United States	2010	Cabanillas and cols.	Single center	Sorafenib Sunitinib	15 DTC	1st line or more	19	Yes: Log Tg
Italy	2013	Marotta and cols.	Single center	Sorafenib	17	1st line	9	Yes: Tg levels and Tg response to treatment, baseline FDG-PET
France	2014	Massicotte and cols.	Multicenter	Sorafenib Sunitinib Vandetanib	45 DTC (17 MTC)	1st line or more	7.0 (1st line DTC)	No
Turkey	2015	Benekli and cols.	Unclear (Turkish Ministry of Health database)	Sorafenib	14 DTC (16 MTC)	Unclear	21.3 (DTC group)	No
France	2017	Berdelou and cols.	Multicenter	Lenvatinib	75	1st line or more	10	No
Spain	2018	Molina-Vega and cols.	Single center	Sorafenib Lenvatinib Axitinib	17	1st line or more	18	No
Korea	2018	Mijin Kim and cols.	Multicenter	Sorafenib	98	1st line	9.7	Yes: Symptoms, lung-only metastasis, daily maintenance dose, Tg reduction
Switzerland*	2018	Balmelli and cols.	Multicenter	Lenvatinib	13	1st line or more	7.2	Yes: Tg levels (with radiologic response)
Japan	2018	Sugino	Single center	Lenvatinib	29	1st line or more	24.3	Symptom
Korea	2019	Kim and cols.	Multicenter	Sorafenib	85	1st line or more	14.4	Yes: Small tumor size, long doubling time
Japan	2019	Suzuki and cols.	Single center	Lenvatinib	26	1st line or more	2 year-PFS= 58.4%	Yes: Baseline tumor size and symptoms
Japan	2019	Yamazaki and cols.	Single center	Lenvatinib	36	1st line or more	Full Dose: 696 days Low Dose: not reached	No
Korea	2019	Lee and cols.	Multicenter (11)	Lenvatinib	67	1st line or more	5.1	Yes: Rapidly PD with shorter initial tumor doubling time
Italy	2019	Locati and cols.	Multicenter (16)	Lenvatinib	94	1st line or more	10.8	No
Argentina	2019	Jerkovich and cols.	Single center	Sorafenib Lenvatinib	22	1st line or more	31.5 (16.5 -1st line only)	No
Japan	2019	Iwasaki and cols.	Multicenter	Sorafenib Lenvatinib	56	1st line	Median treatment duration: Sorafenib 5.1 Lenvatinib 14.1	Yes: Pulmonary metastasis as target lesion
Portugal	2019	Santos and cols.	Single center	Sorafenib Sunitinib	28	1st line or more	10.8 (1st line sorafenib)	No
China	2020	Cheng and cols.	Single center	Sorafenib	72	1st line	17.6	Yes: Hand-foot syndrome, Well DTC, ECOG PS ≤ 2, biochemically nonineffective response, lung-only metastasis, and absence of bone metastasis

Country	Year	Authors	Number of centers	Drugs	Number of subjects	1st line MKI or more	Median PFS (months)	Prognostic Factors
Argentina	2020	Jerkovich and cols.	Multicenter (02)	Lenvatinib	22	1st line or more	13.7	No
Netherlands	2020	Aydermirli and cols.	Multicenter (03)	Lenvatinib	39	1st line or more	9.7	No
Japan	2020	Masaki and cols.	Single center	Lenvatinib	42	1st line or more	13.8	No
Brazil	2020	Treistman and cols.	Single center	Sorafenib	44	1st line	24	FDG uptake on target lesions on PET-CT, higher SUV presence of lung-only metastasis and lower Tg during treatment

TTg: thyroglobulin; DTC: differentiated thyroid cancer; MTC: medullary thyroid cancer; PFS: progression free survival; OS: overall survival; RAI: radioiodine.

71% (30% PR and 41% SD) and Iwasaki and cols.'s 75.0% disease control rate (PR plus SD) (14,34).

Three of our patients did not receive RAI due to unresectable disease and large remaining volume of thyroid tissue, similar cases have also been reported in previous cohorts. Santos and cols., Berdelou and cols. as well as Locati and cols. also described in each report patients that did not undergo thyroid surgery before starting MKI therapy due to unresectable tumors (16,22,30). Those patients would not be eligible for previous MKI trials, however in our experience, two of those three patients had clinical benefit of MKI treatment (one PR and one SD).

When we compared groups divided by outcomes (PD on MKI versus no PD) we found no difference regarding age, number of AE or average RAI activity. We also found no difference regarding symptoms at the beginning of MKI treatment and disease progression as some groups have previously reported. Both Suzuki and cols. and Sugino and cols. have reported that tumor-related symptom were prognostic factors for both poorer PFS and OS in Japanese cohorts (29,36). Kim and cols. also found such association in a multicenter Korean cohort (20). This difference in our results could be explained due to sample size or perhaps different studied population. Even though symptomatic disease did not correlate with PD or death outcomes in our study, 65% of patients who had symptomatic disease before starting MKI reported clinical improvement of symptoms some time during treatment. Berdelou and cols. also described that 52% of their 44 patients with initial symptoms related to DTC had clinical improvement of symptoms (16).

Another interesting finding was that presence of lung-only metastasis was associated with no PD ($p = 0.021$) and that patients who did not progress had on average lower Tg during treatment when compared to patients who progressed ($p = 0.049$). Kim and cols. also described association between lung-only metastasis and PFS, Cheng and cols. also reported that better PFS and OS were found in patients with lung-only metastasis (17,20).

Several authors also found correlations between Tg levels and response to MKI. First, Cabanillas and cols. reported that lower Log Tg was associated to better radiological response (13). Marotta and cols. described that baseline Tg levels were significantly higher in patients who showed disease progression, as well as correlation between baseline Tg and PFS (14). This group also reported that the decrease in serum Tg levels was significantly greater in patients who achieved clinical benefit. In Balmelli and cols.'s report decrease in Tg levels correlated with radiologic response in 6 evaluated patients (31). In Korean population, 60% Tg reduction was associated with better PFS, and more recently Cheng and cols. biochemically response (decrease Tg, stable Tg or increases of under 25%) independently predicted PFS and OS (17,20).

As of major interest, risk factors for cancer-specific mortality was deeply explored. Our group found no difference regarding age, gender, PD site, number of AEs, symptoms at the beginning of MKI therapy or mean RAI activity. Patients who died had larger primary tumor size ($p = 0.035$) and higher incidence of glucose uptake on target lesions on PET-CT ($p = 0.023$). Our group also showed that patients who evolved with PD had a higher incidence of FDG uptake on target lesions on PET-CT when compared to patients who did not progress ($p = 0.033$) and higher maximum SUV on PET-CT on target lesions ($p = 0.042$). Association between

PET-CT findings and response to MKI treatment in RAIR DTC patients is in line with previous reports by Marotta and cols. (14). In their work, baseline average SUVmax was significantly higher in patients who showed disease progression compared with responding subjects, however no significant correlation with PFS was found. Kim and cols. more recently described that the presence of FDG-PET uptake did not affect PFS in his cohort (21). We believe the use of PET-CT in MKI treated RAIR DTC patients should be further analyzed in larger cohorts since we found it as useful in clinical practice.

Regarding safety, most patients presented side effects during MKI treatment. Similar to previous trials, the majority of AE were low grade (11,12). However, in 50% of cases, reducing medication dosage was necessary at some point to manage side effects, similarly to Santos and cols. and Balmelli and cols. (30,31). In 25% of cases the drug was eventually suspended due to AE, also reported by Kim and cols.'s (23% permanent discontinuation) – but higher than reported by Jerkovich and cols. and Benekli and cols. with only 1 patient in each series permanently suspending sorafenib (18,21,25). Only one secondary cutaneous neoplasia was found in our cohort. Squamous cell carcinoma was found in 7 out of 207 sorafenib treated patients in DECISION trial, and other colleagues reported similar occurrences (11,13,18). The fact that almost every patient will experience AE at one point during MKI treatment and that AE might interfere with ongoing treatment highlights the importance of an experienced assistant team to manage such drugs.

Our work, however, has limitations. As a retrospective cohort, we had some cases of loss of follow-up. In addition, when we perform chart analysis, we have come across some missing data. The limited size of our sample may limit conclusions and reduce statistical power. As any study in a real-life setting, there are often difficulties in scheduling and performing exams, poor adherence to treatment, missed appointments and other factors that may interfere in some way with the results.

Nevertheless, is the first Brazilian report and one of the few subcontinental cohorts validating findings in other populations and demonstrating safety and efficacy of the use of MKI in RAIR-DTC. Our findings also corroborate previous authors that found presence of lung-only metastasis, absence of FDG uptake on target lesions on PET-CT, lower maximum SUV on PET-CT and lower Tg during treatment associated with better outcomes in RAIR DTC patients treated with MKI.

In conclusion, our analysis demonstrates that the use of MKI drugs in patients with advanced RAIR DTC is a safe and effective therapeutic approach and results were consistent with international literature data, with median PFS of 24 months (10.2-37.7) and 72.7% clinical benefit from MKI treatment. We were able to identify absence of FDG uptake on target lesions on PET-CT, lower maximum SUV on PET-CT, presence of lung-only metastasis and lower Tg during treatment as possible prognostic markers.

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Effect of beinaglutide treatment on weight loss in Chinese patients with type 2 diabetes mellitus and overweight/obesity

Guiying Wang[1]*
https://orcid.org/0000-0001-8072-0240

Peng Wu[2]*
https://orcid.org/0000-0002-8986-0204

Yan Qiu[2]
https://orcid.org/0000-0002-4540-3183

Xin Dong[2]
https://orcid.org/0000-0002-3876-1767

Yingbin Wang[1]
https://orcid.org/0000-0001-8575-065X

Yanjun Chi[1]
https://orcid.org/0000-0003-3428-7225

Fengjuan Zhang[1]
https://orcid.org/0000-0003-4029-1482

Yinyu Li[1]
https://orcid.org/0000-0001-7808-7634

Jimin Zhang[1]
https://orcid.org/0000-0001-5357-5674

Zhengli Huang[1]
https://orcid.org/0000-0003-2044-2802

Xifeng Du[2]
https://orcid.org/0000-0003-0070-8677

Zhiqiang Du[2]
https://orcid.org/0000-0003-0686-1891

[1] Department of Endocrinology and Metabolism, The First Affiliated Hospital of Datong University, Datong, Shanxi, China
[2] Shanghai Benemae Pharmaceutical Corporation, Shanghai, China

*Co-first Author

ABSTRACT

Objective: To evaluate the effect of beinaglutide on weight loss and plasma protein patterns of inflammation/obesity relevant cytokines and biomarkers. **Materials and methods:** This study involved 36 adult patients with a body mass index (BMI) of ≥ 24 kg/m² and T2DM. Beinaglutide was administered for three months. Changes in body weight, fasting plasma glucose (FPG) level, 2 h postprandial plasma glucose (2h-PG) level, glycosylated hemoglobin (HbA1c) level, BMI and visceral and subcutaneous fat areas were measured at baseline and after three months of treatment. In addition, relevant inflammation/obesity cytokines and biomarkers were measured. **Results:** After three months, beinaglutide treatment led to significant changes, including in body weight, BMI, FPG level, HbA1c level, visceral and subcutaneous fat areas. In addition, serpin E1, leptin, C-reaction protein (CRP) and tumor necrosis factor-α (TNF-α) also decreased significantly. The plasma protein concentrations of CRP (Log2 transformed) were found to be positively correlated with the percentage of weight loss (R = 0.514 and p-value = 0.021). **Conclusion:** Beinaglutide treatment resulted in weight loss, plasma glucose control and anti-inflammatory effects in patients with T2DM and overweight/obesity. Arch Endocrinol Metab. 2021;65(4):421-7

Keywords
T2DM; overweight; obesity; GLP-1

Correspondence to:
Zhiqiang Du
Shanghai Benemae Pharmaceutical Corporation
916 Ziping Road, 201321, Shanghai, China
duzhiqiang@benemae.com

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INTRODUCTION

The number of people with overweight/obesity has been steadily increasing. Obesity has been recognized as one of the greatest public health concerns worldwide, especially in developed countries (1). A study conducted in different parts of China revealed that the prevalence of obesity is increasing (2). Obesity

increases the morbidity of several chronic diseases and also increases the mortality of cardiovascular diseases, diabetes, cancers and musculoskeletal disorders (3-7). Among Chinese patients (aged 35-74 years) with type 2 diabetes mellitus (T2DM) in 2010, 28.3% and 10.1% of males and 31.3% and 16.8% of females were considered overweight and obese, respectively. It is reported that approximately 3.32 million T2DM events were attributable to overweight and obesity (8).

Several GLP-1 receptor agonists (GLP-1RA) have been promoted for the treatment of T2DM and have multiple potential effects on hyperglycaemia, cardiovascular and liver disease (9,10). Additionally, the mechanism by which GLP-1RA treatment leads to weight loss has been reported (11). Among these GLP-1RAs drugs, beinaglutide is the only prandial GLP-1 (7-36) receptor agonist with a 100% protein sequence identity to human 7-36 GLP-1. A retrospective observational real-world study reported that beinaglutide significantly reduced the bodyweight of the patients with T2DM and obesity while lowering the plasma glucose and glycosylated hemoglobin (HbA1c) levels (12).

However, inflammation/obesity relevant cytokines and biomarkers from plasma in patients with T2DM treated with beinaglutide are still unknown. Our study aimed to evaluate the effect of beinaglutide on weight loss and plasma protein patterns of inflammation/obesity relevant cytokines and biomarkers.

MATERIALS AND METHODS

Study design and patients

This study was a single-centre, prospective, open-label, self-controlled real-world study (RWS) involving 36 patients with overweight/obesity and T2DM. Patients were recruited from the First Affiliated Hospital of Shanxi Datong University. All patients gave their informed consent before receiving beinaglutide treatment. This study was approved by the Ethics Committee of the First Affiliated Hospital of Shanxi Datong University in China and conducted in accordance with the ethical guidelines of the Declaration of Helsinki (1975).

The inclusion criteria were adult patients (≥18 years old) with a body mass index (BMI) of ≥ 24 kg/m^2 and a diagnosis of T2DM according to the WHO 1999 criteria. The exclusion criteria were patients with clinically significant cardiac, central nervous system, rheumatic or cancer diseases, females who were pregnant or planning to become pregnant, patients enrolled in another clinical trial within the past three months, patients who used a GLP-1 receptor agonist or weight loss drug in the past three months, patients with long-term use of glucocorticoids that led to being overweight/obese, acute diabetic complications, infections or other endocrine diseases, patients with a history of pancreatitis, cancer of the pancreas, type 2 multiple endocrine neoplasia syndrome, or medullary thyroid carcinoma, or patients with inflammatory intestinal diseases or diabetic gastric paretitis.

Beinaglutide injections were administered twice per day before meals, with a starting dose of 0.06 mg per injection each week, climbing to 0.1 mg per injection for three months. Bodyweight (BW), BMI, visceral fat area (VFA), subcutaneous fat area (SFA), waist circumference (WC), HbA1c level, fasting plasma glucose (FPG) level, 2 h postprandial plasma glucose (2h-PG) level, heart rate and blood pressure were measured at baseline and after three months. Besides, plasma was collected for cytokines and biomarkers detection before and after three months of treatment with beinaglutide. The primary end-point was weight loss after three months compared to the baseline. The secondary end-points were changes in FPG, 2h-PG, HbAlc, BMI, VFA and SFA after three months compared to the baseline.

Cytokines and biomarkers measurement

Plasma was separated within 24 h from the patients at baseline and three months later and then stored at -80 °C for future analysis. The Human Obesity Premixed Mag Luminex Performance Assay Kit (FCSTM08-10, R&D Systems) was used to analyse ten inflammation/obesity related plasma cytokines and biomarkers including Adiponectin/Acrp30, C-Reactive Protein/CRP, Complement Factor D/Adipsin, Serpin E1/PAI-1, CCL2/JE/MCP-1, IL-6, IL-10, Leptin/OB, resistin and TNF-α which sensitivities were 6.4 pg/mL, 1.4 pg/mL, 0.16 pg/mL, 1.8 pg/mL, 0.36 pg/mL, 0.13 pg/mL, 7.69 pg/mL, 0.85 pg/mL, 0.20 pg/mL and 0.60 pg/mL, respectively. The panel is a Luminex system that uses a fluorescent bead system and CV (coefficients of variation) of the cytokines/biomarkers are all below 20%. All assay procedures were performed as described by the manufacturer.

Quantification of visceral and subcutaneous fat area

Abdominal bioelectrical impedance analysis (BIA) (OMRON, HDS-2000 DUALSCAN) was used to estimate VFA and SFA (13). The VFA was calculated by calculating the total sectional area of the abdomen with the BIA between the umbilicus and the back.

Statistical analyses

Statistical analysis was conducted and boxplots were generated using R software (version 3.6.1). A normality test of the original data was performed using shapiro.test function using R software. The cytokines and biomarkers data were log2 transformed to check for normality. Baseline and treatment data from the clinical data set were analysed by paired t-tests based on the normality of the original data. Paired one-side Wilcoxon tests with an alternative hypothesis of "greater" were used for non-parametric tests of the non-parametric plasma cytokines and biomarkers data set. The p-value cut-off was set at 0.05. Correlation analysis was performed using the cor.test function in R software using the Pearson method. Weight loss (weight change) percentage was equal to (baseline body weight (kg)-treatment body weight (kg))/baseline body weight (kg).

RESULTS

Baseline characteristics and clinical outcomes

A total of 36 participants complied with the criteria for this study. Twenty-four males and twelve females, with an overall mean age of 46.25 (±13.55) years. Baseline characteristics and clinical outcomes after three months of treatment with beinaglutide are shown in Table 1 and Figure 1. In brief, bodyweight related physical indices such as BW, BMI, VFA, SFA, WC and hip circumference were reduced significantly after three months (p-value < 0.05). There were 63.9% (23/36) who lost 3% or more in weight, and 41.7% (15/36) lost more than 5% (data not shown). HbA1c and FPG levels were also significantly reduced, while heart rate, systolic blood pressure and diastolic blood pressure did not. These results suggest that patients with T2DM and overweight/obesity could benefit from beinaglutide for weight loss and plasma glucose control.

Inflammation/obesity related cytokines and biomarkers

Plasma concentrations of the panel of ten cytokines and biomarkers were quantified using a Luminex detection platform. Changes compared to baseline and three months of treatment are shown in Table 2 and Figure 2.

Table 1. Summary of patient characteristics before and after treatment with beinaglutide

	Number	Baseline	Treatment	p-value
Demographics				
Gender	36	12 F/24 M	12 F/24 M	NA
Age (y)	36	46.25 (±13.55)	46.25 (±13.55)	NA
Obesity index				
Weight (kg)	36	88.97 (±11.32)	85.18 (±11.45)	**<0.001**
BMI (kg/m^2)	36	31.63 (±3.55)	30.61 (±3.32)	**<0.001**
HR (bpm)	35	84.06 (±14.01)	79.63 (±13.69)	0.09115
SBP (mmHg)	35	139 (±16.52)	138.23 (±17.19)	0.68123
DBP (mmHg)	35	81.42 (±11.21)	81.77 (±12.83)	0.93020
Hip circumference (cm)	36	108 (±7.02)	104.68 (±7.16)	**0.00104**
Waist circumference (cm)	36	103.74 (±7.51)	98.71 (±7.93)	**<0.001**
VFA (cm^2)	36	158.81 (±23.11)	120.6 (±38.79)	**<0.001**
SFA (cm^2)	36	281.43 (±65.75)	267.64 (±62.88)	**0.02626**
Plasma Glucose test				
HbA1c (%)	24	8.75 (±2.31)	7.38 (±1.90)	**<0.001**
FPG (mmol/L)	20	10.01 (±3.96)	8.62 (±3.87)	**0.008**
2h-PG (mmol/L)	18	14.44 (±4.58)	12.20 (±4.65)	**0.012**

F/M: female/male; BMI: body mass index; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; VFA: visceral fat area; SFA: subcutaneous fat area; HbA1c, haemoglobin A1c; FPG: fasting plasma glucose; 2h-PG: 2-hour plasma glucose. Data are shown as Mean ± SD.

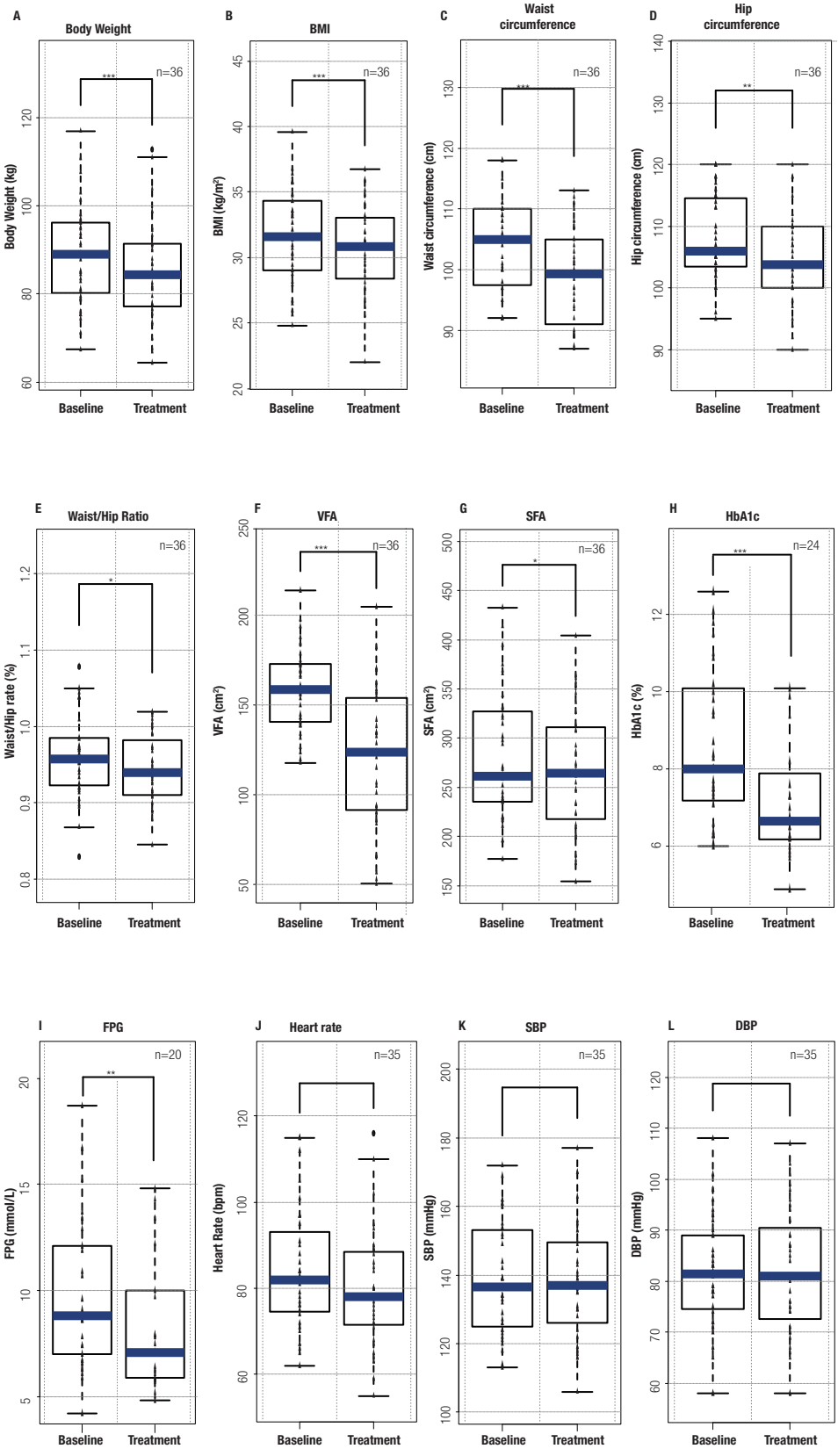

Figure 1. Comparison of baseline and treatment of patients receiving beinaglutide in body weight (A), body mass index (B), waist circumference (C), hip circumference (D), waist/hip rate (E), visceral fat area (F), subcutaneous fat area (G), HbA1c (H), fasting plasma glucose (I), heart rate (J), systolic blood pressure (K) and diastolic blood pressure (L). * $p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Serpin E1, TNF-α, Leptin and CRP were significantly decreased after treatment compared with baseline levels (p-value < 0.05; Figure 2). The other five cytokines, adiponectin, CCL2, complement factor D, IL-10 and resistin, were not reduced, while IL-6 failed to be detected due to an extremely low signal. Correlation analysis showed that the Log2 transformed plasma protein concentrations of CRP was positively correlated with the percentage of weight loss (R = 0.514, p-value = 0.021, Table 3).

DISCUSSION

Beinaglutide was approved as a novel drug for diabetes treatment and the clinical benefit of weight loss and plasma glucose control has also been reported (12). However, the protein pattern of plasma cytokines and biomarkers are still unclear. This study found a mean weight loss of 3.8 kg and 4.3% mean weight loss percentage after three months of treatment. Moreover, BMI, WC, hip circumference, visceral fat, and subcutaneous fat were also significantly decreased

Table 2. Changes of inflammation/obesity related plasma cytokines and biomarkers before and after treatment with beinaglutide

	Baseline	Treatment	p-value
Adiponectin (ng/mL)	6046.23 (±4093.7)	6739.87 (±4602.93)	0.938
CCL2 (pg/mL)	121.84 (±49.87)	105.81 (±29.64)	0.084
CRP (ng/mL)	7627.52 (±8437.05)	4197.02 (±6811.5)	**0.044**
Complement Factor D (ng/mL)	6191.26 (±1483.5)	6531.85 (±1774.22)	0.554
IL-10 (pg/mL)	2.07 (±0.25)	2.05 (±0.25)	0.462
Leptin (ng/mL)	23.15 (±16.76)	18.45 (±12.63)	**0.003**
Resistin (pg/mL)	8.48 (±3.52)	9.24 (±4.09)	0.862
Serpin E1 (ng/mL)	48.02 (±24.2)	25.1 (±13.15)	**0.000**
TNF-α (pg/mL)	4.08 (±3.05)	3.26 (±2.55)	**0.005**
IL-6	NA	NA	NA

NA: no available data.

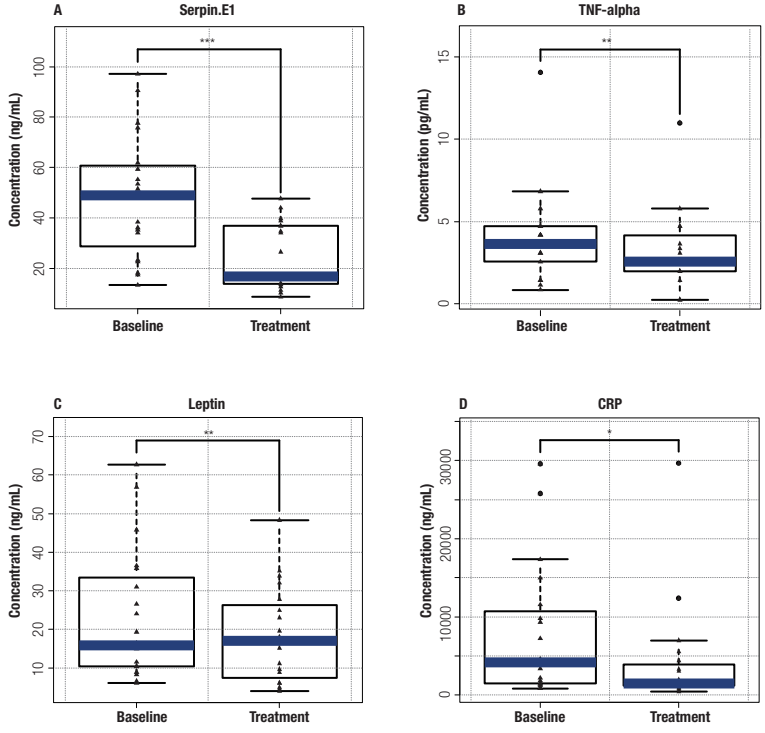

Figure 2. Comparison of baseline and treatment of patients receiving beinaglutide in 4 inflammation/obesity related cytokines/biomarkers. (A) Serpin E1, (B) TNF-α, (C) Leptin, (D) C-reactive protein. * p < 0.05; **p < 0.01; ***p < 0.001.

Table 3. Correlation analysis between inflammation/obesity related plasma cytokines/biomarkers and weight loss percentage

Cytokines/biomarkers	R	p-value	R-log2	p-value_log2
CRP	0.398	0.082	**0.514**	**0.021**
Adiponectin	-0.131	0.583	-0.153	0.518
TNF-α	-0.042	0.871	-0.044	0.868
Adipsin	0.109	0.724	0.101	0.742
IL-10	0.061	0.799	0.059	0.806
CCL2	-0.025	0.918	-0.032	0.894
Resistin	**0.546**	**0.013**	0.442	0.051
Serpin E1	0.033	0.89	-0.008	0.973
Leptin	0.176	0.457	0.196	0.407
IL-6	NA	NA	NA	NA

R (and p-value): correlation (and p-value) between weight loss percentage and plasma cytokines/biomarkers concentrations; R-log2 (and p-value_log2): correlation (and p-value) between weight loss percentage and log2 transformed cytokines/biomarkers concentration; NA: no available data.

after beinaglutide treatment but not heart rate, diastolic blood pressure or systolic blood pressure (SBP). In addition, HbA1c, FPG and 2h-PG levels were also decreased. It has been reported that heart rate and SBP are affected by other GLP-1RA drugs with a long half-life and continuously activation of GLP-1R (14,15). Therefore, the results of this study indicate the advantages of beinaglutide as a GLP-1 homolog with 100% protein sequence identity to human natural GLP-1 and short half-life (30 min) (16,17).

Abdominal visceral fat and subcutaneous fat are independent risk factors for cardiovascular diseases and metabolic syndrome (18,19). In this study, visceral fat decreased significantly by approximately 24% on average, while subcutaneous fat decreased by about 4% on average after three months of beinaglutide treatment. This finding suggests that beinaglutide primarily reduced body weight through visceral fat, which could be critical for patients with T2DM to reduce the risk of the related diseases.

In addition, we tested the exploratory cytokines and biomarkers of inflammatory or obesity to determine the related factors associated with weight loss after beinaglutide treatment. Serpin E1 is highly expressed in the plasma of patients with obesity and diabetes, indicating a link between them (20). Our data showed that serpin E1 was significantly downregulated in the plasma of the patients with T2DM and overweight/obesity after three months of treatment with beinaglutide. This finding suggests that the downstream signalling pathway of the GLP-1 receptor might be associated with the regulation of Serpin E1 (21,22).

Obesity is considered a chronic inflammatory disease. Macrophage migration in patients with obesity infiltrates into the vicinity of fat cells, leading to the secretion of inflammatory cytokines, thereby upregulating the expression of TNF-α and resistin (23). In this study, TNF-α was significantly reduced, suggesting the anti-inflammatory effect of beinaglutide. A parallel study has reported that inflammatory factors such as TNF-α decreased after GLP-1 analog treatment in patients with diabetes and obesity (24,25).

It has been reported that leptin decreased after treatment with diet control or GLP-1 drugs in patients with T2DM with effective or ineffective weight loss (25,26). The serum leptin level in patients with obesity was higher than that in the normal weight group, and a decrease in body weight led to a decrease in leptin levels (27,28). The results in our study are consistent with these studies, thus illustrating the importance of leptin in weight loss.

CRP is a widely investigated biomarker of inflammation in the pathogenesis of several chronic diseases such as cardiovascular disease and diabetes. It has been reported that a decrease in CRP is associated with direct weight loss (29). Mazidi's study showed that the level of CRP significantly decreased after treatment with GLP-1 RAs (30). The results of our study verified this finding.

Our research has several limitations. The limited sample size was an inevitable problem in our study, and the study would be informative if there were more data. However, these findings provide a foundation for future clinical research. Next, we will conduct a further study with a large population to validate our findings.

In summary, this study is the first to report the plasma protein pattern of inflammation/obesity related

cytokines and biomarkers in Chinese patients with T2DM and overweight/obesity who were treated with beinaglutide. Beinaglutide also decreased bodyweight, as well as the plasma glucose and inflammatory levels. Furthermore, baseline level of CRP is related to the weight loss percentage of beinaglutide treatment in patients with T2DM and overweight/obesity.

Authorship: G.W. and Z.D. designed and supervised the study, P.W. performed the experiments, Y.W., Y.C., F.Z., Y.L. and J.Z. enrolled patients, Z.H. and X.D. collected samples, P.W. and X.D. analyzed the data, P.W. wrote the manuscript, P.W., Y.Q. and X.D. revised the manuscript.

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Thyroglobulin/thyrotropin ratio for predicting long-term response in differentiated thyroid carcinoma: a retrospective study

[1] Departamento de Clínica Médica, Faculdade de Medicina de Botucatu, Universidade Estadual Paulista (Unesp), Botucatu, SP, Brasil
[2] Departamento de Oftalmologia, Otorrinolaringologia e Cirurgia de Cabeça e Pescoço, Faculdade de Medicina de Botucatu, Universidade Estadual Paulista (Unesp), Botucatu, SP, Brasil
[3] Departamento de Patologia, Faculdade de Medicina de Botucatu, Universidade Estadual Paulista (Unesp), Botucatu, SP, Brasil
[4] Departamento de Medicina Nuclear, Faculdade de Medicina de Botucatu, Universidade Estadual Paulista (Unesp), Botucatu, SP, Brasil

Adriano Francisco De Marchi Junior[1]
https://orcid.org/0000-0001-9939-3569

Ana Bárbara Trizzotti de Macedo[1]
https://orcid.org/0000-0002-2334-1639

Carlos Segundo Paiva Soares[2]
https://orcid.org/0000-0003-0121-9533

Fernanda Bolfi[1]
https://orcid.org/0000-0002-1444-592X

Mariana Riello Gomes Iessi[1]
https://orcid.org/0000-0002-9235-5209

Cristiano Claudino de Oliveira[3]
https://orcid.org/0000-0001-6682-5230

Katia Hiromoto Koga[4]
https://orcid.org/0000-0001-6223-7263

Sonia Marta Moriguchi[4]
https://orcid.org/0000-0001-5834-2714

José Vicente Tagliarini[2]
https://orcid.org/0000-0002-0869-724X

Gláucia Maria Ferreira da Silva Mazeto[1]
https://orcid.org/0000-0003-2129-7256

Correspondence to:
Gláucia Maria Ferreira da Silva Mazeto
Departamento de Clínica Médica, Faculdade de Medicina de Botucatu, Universidade Estadual Paulista (Unesp)
Av. Prof. Mário Rubens Guimarães Montenegro, s/n
18618-970 – Botucatu, SP, Brasil
g.mazeto@unesp.br

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ABSTRACT

Objective: Thyrotropin-stimulated thyroglobulin (STg) after total thyroidectomy is a prognosis marker for differentiated thyroid carcinoma (DTC). As Tg level is influenced by thyrotropin (TSH), perhaps the STg/TSH ratio is also a prognosis marker for these tumours. We aimed to compare STg/TSH ratio and first STg level in differentiated thyroid carcinoma patients for their ability to predict the long-term response to initial treatment. **Subjects and methods:** This retrospective study evaluated data from 181 DTC patients for first (1st) STg and STg/TSH ratio, at 1-3 months post-total thyroidectomy and before iodine-131 therapy, according to response to initial therapy [Excellent/Indeterminate or Incomplete (Biochemical/Structural)] observed at final evaluation, and with the survival time with excellent/indeterminate response. **Results:** Cases with incomplete response presented higher STg level [225.13 ± 585.26 ng/mL *versus* (*vs*) 20.4 ± 192.9 ng/mL; p < 0.001] and STg/TSH ratio (3.01 ± 7.8 *vs* 0.27 ± 2.58; p < 0.001). Cutoffs of 5 ng/mL for STg and 0.085 for STg/TSH displayed sensitivities of 76.7% and 76.9%, and specificities of 79.2% and 82.6%, respectively, in predicting response to therapy. Values below these cutoffs were associated with longer survival time in excellent/indeterminate response (140.4 *vs* 15.9 and 144.6 *vs* 15.9 months, respectively). **Conclusion:** STg/TSH ratio has a similar performance to the 1st STg in predicting long-term response to initial therapy. Arch Endocrinol Metab. 2021;65(4):428-35

Keywords
Thyroid neoplasms; thyroglobulin; prognosis

INTRODUCTION

Differentiated thyroid carcinomas (DTC) make up almost all malignant thyroid neoplasias and have been increasing in incidence (1). The most frequent DTC's are papillary carcinomas (PC) accounting for more than 80% of cases with follicular carcinomas (FC) corresponding to between 10 and 13% (2,3).

DTC's have low lethality rates and slow indolent growth. Although 10 year survival rates are around 80%-95%, some cases have more aggressive behaviour and recurrence levels up to 35% indicating a cautious approach and long-term clinical follow-up (4-8). For a long time the standard treatment for these tumours was total thyroidectomy (TT) followed by iodine-131

(^{131}I) therapy and thyrotropin (TSH) suppression with levothyroxine (2). More recently, more conservative surgical therapies have been proposed along with less rigorous follow-up strategies depending on tumour presentation and behaviour, and case evolution expectations (2).

Thus, efforts have been made to detect early markers of patient evolution in order to better control the need for additional treatment to thyroidectomy and more rigorous long-term follow-up. Several individual factors have been reported as prognosis indicators; these include patient age and lymph node involvement (5). Additionally, staging systems applied after initial treatment have been used for this purpose, the most common being from the American Joint Committee on Cancer (AJCC/TNM) (2). As this system evaluates the risk of death from neoplasia, and as mentioned above, DTC is characterised by its low mortality rates, the American Thyroid Association (ATA) proposed a recurrence risk stratification system, classifying cases as low, intermediate or high risk (2). Although this system is very useful, it does not evaluate long-term case evolution according to treatment given. Thus, the latest ATA guidelines suggest a new more dynamic classification system based on therapeutic response to initial treatment (2).

Although all these systems have useful proven tools in evaluating risk of death and recurrence, they do present some complexity as many factors need to be assessed. It would therefore be very interesting if there was a unique marker which could be used at the start of follow-up to predict which patients need more aggressive treatment. In this context emerges the role of measuring thyroglobulin (Tg) serum level which has already been demonstrated as a prognosis predictor in DTC (9-12). Its levels, straight after initial treatment, can help determine the presence of persistent or recurrent disease and represent an independent prognosis factor for treatment success (11).

After TT, Tg seems to reach its lowest level at around three to four weeks (2), and its levels are influenced by various factors, including the presence of anti-Tg antibodies (TgAb) (6,13,14), the remaining quantity of thyroid tissue, the presence of metastatic lesion, time since surgery, and characteristics inherent to the test used for taking measurements (2,15). Also, TSH level at the time of Tg measurement could have an impact on marker concentration (2,15). In fact, although that both basal Tg (during levothyroxine treatment) and stimulated Tg (by high TSH levels; STg) can be used (2), in about 20% of patients with undetectable basal Tg, the marker is elevated when TSH is higher, which could indicate a worse prognosis (16,17). Thus, STg, obtained both with endogenous TSH and with recombinant TSH use, could be more sensitive than basal Tg (17).

To measure STg under stimulation with endogenous TSH, it is generally necessary to suspend levothyroxine for three to four weeks, aiming at a significant increase in TSH. Unfortunately, the ideal TSH level to stimulate Tg production by the remaining thyroid/neoplastic cells has not yet been fully established. Concentrations above 30 mU/L seem to be necessary, however, levels between 60 and 90 mU/L would be more reliable for evaluating the marker (18). It is also difficult to compare different STg measurements and the marker level evolution considering the possibility that TSH concentration may be different in each sample. For example, would the same STg values obtained with TSH levels of 31 or 90 mU/L have the same meaning? In this sense, considering pituitary hormone level, the STg/TSH ratio could have a greater potential for comparison and be a better predictor of therapeutic success than the isolated STg level. The STg/TSH ratio has already proved to be a reliable marker in predicting ^{131}I ablative/therapeutic success (11). In fact, when obtained in the pre-ablative period, this ratio seems valuable in predicting both the ablative success (19), and the presence of metastases (15). No references were found on using the STg/TSH ratio to assess long-term case evolution. Thus, this study aimed to compare STg/TSH ratio and first STg level in their ability to predict long-term response to initial therapy.

SUBJECTS AND METHODS

This study was approved by the Botucatu Medical School Ethics Committee (CAAE no. 83473918.6.0000.5411; Reference no. 2.532.645). This retrospective study compared STg/TSH ratio and first post-thyroidectomy STg level (1st STg) for capacity to predict therapeutic response in DTC patients at final evaluation and during follow-up using classification from the latest ATA guidelines (2). Data were collected from medical records of DTC patients evaluated in a tertiary hospital.

Patients

We evaluated 278 DTC patients followed at a specialised out-patient clinic and selected 181 cases (65.1%) according to the following criteria. Inclusion criteria

were: cases with anatomopathological DTC diagnosis, submitted to this service's standardised initial treatment between 2001 and 2015 (the period where a standard Tg measuring method was maintained), which at that time consisted of TT followed by ^{131}I therapy (DTI); who had Tg, TgAb and TSH results evaluated 1 to 3 months after TT and before DTI; and who had had clinical, laboratory, cervical ultrasound exam follow-up for at least 24 months after initial treatment. Exclusion criteria were: patients who presented positive TgAb; and those with TSH and Tg levels measured from different samples and by different methodologies.

Variables of interest and evaluated outcomes

The main variables of interest in this study were 1st STg concentrations evaluated between 1 to 3 months post-TT but before DTI, and the ratio between 1st STg and TSH (STg/TSH). Evaluated outcomes were response to treatment at final evaluation and survival time maintained in excellent/indeterminate response. Response to therapy was assessed according to latest ATA therapy response guidelines: excellent, biochemical incomplete, structural incomplete, and indeterminate response (2). According to this classification, patients were categorised as excellent/indeterminate response or incomplete (biochemical and/or structural) response.

The following patient data were also collected: gender; referred race, white or non-white; age a diagnosis in years; neck dissection performed; anatomopathological diagnosis, according to previous recommendations (20); staging with regard to risk of death, according to AJCC/TNM 7th edition classification system (21); and with regard to ATA predicted risk of recurrence (low, intermediate, or high) (2); result of 1st whole body scan (WBS; considered positive when with cervical or distant uptake after administration of a ^{131}I tracing dose), total accumulated therapeutic ^{131}I dose; response to therapy 1 year after initial treatment, and follow-up time in months.

Methods

Treatment and follow-up of DTC patients was performed during the data collection period for this study, as previously reported (11). Briefly, this included TT, reassessment 1 to 3 months after surgery with STg measured by endogenous TSH (1st STg), WBS, and neck ultrasound (US). Patients subsequently received DTI and were submitted to post-dose WBS about 5 days after treatment. A new evaluation was performed 1 year after DTI with diagnostic WBS, cervical US, and STg and TSH levels. During follow-up, patients were re-evaluated every four to six months with clinical examination and measurements of free thyroxin (FT4), TSH, Tg and TgAb, as well as annual neck ultrasound. If findings suggested persistent or recurrent disease, other imaging tests such as computed tomography, magnetic resonance and positron emission computed tomography (PET-CT) were requested, and if necessary cytohistological exams.

Measurements of FT4, TSH and Tg were performed the Service's Clinical Laboratory using chemiluminescence (DPC, Los Angeles, CA, USA), with the following reference values (RV): 0.80-1.90 ng/dL, 0.40-4.0 µIU/mL and 0.83-68.0 ng/mL, respectively. Analytical Tg sensitivity was 0.2 ng/mL, while functional sensitivity was 0.9 ng/mL (for levels higher than 2 ng/mL). TgAb was measured at the same laboratory by chemiluminescence (Immulite 2000, Siemens, Llanberis, Gwynedd, United Kingdom), with manufacturer RV of ≤ 40 UI/mL, above which it was considered positive.

Statistical analysis

Collected data were tabulated in Excel® (Microsoft Corporation, USA) and submitted to statistical analysis using SAS v9.4 software. Qualitative variables were expressed as frequencies and percentages and evaluated using the Chi-squared and Fisher Exact tests. Quantitative variables were expressed and means and standard deviations and evaluated by the Student t test. In general, a 5% ($p < 0.05$) significance level was adopted. However, variables with $p \leq 0.15$ were submitted to multivariate logistic regression with incomplete (biochemical/structural) response to initial therapy as the response variable. This higher than usual level of significance (0.15) was used to minimize the risk of neglecting important variables for the outcome. ROC (receiver-operating characteristic) curves were constructed for the 1st STg and STg/TSH ratio to establish cutoffs which could predict incomplete therapeutic (biochemical/structural) response, with respective area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Considering these cutoffs, Kaplan-Meyer curves were also constructed to evaluate survival time in excellent/indeterminate response.

RESULTS

Clinical, histopathological, therapeutic, and evolution data of the studied cases can be found in Table 1. The majority (n = 158; 87.3%) were female and declared race white (n = 169; 93.4%). Seventy-five patients (41.4%) were submitted to lymph node dissection. The most frequent histological type was CP (n = 163; 90.1%), with 117 cases (64.6%) having presented TNM Stage I and 99 (54.7%) displaying low risk of recurrence. The majority of cases (n = 170; 94.4%) presented positive at 1st WBS, and of these most had only had neck uptake (n = 163; 95.9%). Accumulated mean DTI [±standard deviation (SD)] was 158.77 (±56.92) mCi. Mean (±SD) values of 1st STg and STg/TSH ratio were 35.11 (±245.29) ng/mL and 0.47 (±3.28), respectively. One hundred and sixteen patients (64.1%) presented an excellent response 1 year after initial treatment, while 120 (66.3%) presented this same response at final evaluation, with a mean (±SD) follow-up time of 87.24 (±44.94) months (median = 76, minimum = 24, maximum = 188 months). Twenty-eight cases (15.5%) presented incomplete (biochemical or structural) response at some time during follow-up.

Cases with incomplete (biochemical or structural) response at final evaluation were compared with those with excellent/indeterminate response (Table 2), with the former group displaying higher serum concentrations of 1st STg [225.13 ± 585.26 ng/mL *versus* (*vs*) 20.4 ± 192.9 ng/mL; p < 0.001] and STg/TSH ratio (3.01 ± 7.8 *vs* 0.27 ± 2.58; p < 0.001). The patients with incomplete response also presented a higher percentage of cases of high risk of recurrence (38.5% *vs* 14.9%; p = 0.078) and received higher accumulated doses of ^{131}I (383.8 ± 286.6 mCi *vs* 168.02 ± 86.51 mCi; p < 0.0001). The groups did not differ in the other evaluated parameters.

In the multivariate analysis, performed with variables where p ≤ 0.15, the type of cancer [for FC; odds ratio (OR) = 5.552; confidence interval (CI): 1.082-28.5; p = 0.04] and accumulated DTI (OR = 1.008; CI: 1.003-1.013; p = 0.001) continued as significant.

ROC curves were constructed to establish cutoff points for 1st STg and STg/TSH values that could predict a higher risk of the patient presenting incomplete response at last evaluation (Figure 1). The cutoff obtained for 1st STg was 5 ng/mL, with AUC of 0.907 (p < 0.001; CI 95%: 0.84-0.97), 76.7% sensitivity, 79.2% specificity, 22.2% VPP, 97.8% VPN and 79% accuracy. For the STg/TSH ratio cutoff was 0.085, with AUC of 0.920 (p < 0.001; CI 95%: 0.87-0.97), 76.9% sensitivity, 82.6% specificity, 25.6% VPP, 97.8% VPN and 82.2% accuracy.

Table 1. Clinical, histopathological, therapeutic, and evolutionary data of the studied population

Data	
Female, n (%)[a]	158 (87.3)
White color reported, n (%)[a]	169 (93.4)
Age (years)[b]	48.45 ± 14.19
Lymph node dissection, n (%)[a]	75 (41.4)
Type of cancer	
Papillary carcinoma, n (%)[a]	163 (90.1)
Classical variant, n (%)[a]	101 (62.0)
Follicular variant, n (%)[a]	51 (31.3)
Oncocytic variant, n (%)[a]	6 (3.7)
Sclerosing variant, n (%)[a]	2 (1.2)
Solid variant, n (%)[a]	3 (1.8)
Follicular carcinoma, n (%)[a]	18 (9.9)
Staging (TNM)	
I, n (%)[a]	117 (64.6)
II, n (%)[a]	25 (13.8)
III, n (%)[a]	24 (13.3)
IV, n (%)[a]	15 (8.3)
Risk of recurrence	
Low, n (%)[a]	99 (54.7)
Intermediate, n (%)[a]	52 (28.8)
High, n (%)[a]	30 (16.6)
Positive 1st WBS, n (%)[a]	170 (94.4)
Cervical uptake, n (%)[a]	163 (95.9)
Cervical and distant uptake, n (%)[a]	7 (4.1)
RIT total dose (mCi)[b]	158.77 ± 56.92
1st STg (ng/mL)[b]	35.11 ± 245.29
STg/TSH ratio[b]	0.47 ± 3.28
Therapeutic response in one year	
Excellent, n (%)[a]	116 (64.1)
Indeterminate, n (%)[a]	50 (27.6)
Incomplete biochemistry, n (%)[a]	3 (1.7)
Incomplete structural, n (%)[a]	12 (6.6)
Incomplete response at some point, n (%)[a]	28 (15.5)
Therapeutic response in the last evaluation	
Excellent, n (%)[a]	120 (66.3)
Indeterminate, n (%)[a]	48 (26.5)
Incomplete biochemistry, n (%)[a]	3 (1.7)
Incomplete structural, n (%)[a]	10 (5.5)
Follow-up time (months)[b]	87.24 ± 44.96

1st STg: first stimulated thyroglobulin; mCi: milicuries; n: number; %: percentage; RTI: radioactive iodine ablation/therapy; TNM: American Joint Commission on Cancer (AJCC) tumor staging system; TSH: thyrotropin; WBS: whole-body scan.
[a] Frequencies and percentages for categorical variables.
[b] Mean ± standard deviation.

Table 2. Comparison between patients with excellent/indeterminate and incomplete response (biochemical and/or structural) in the last evaluation, regarding clinical, laboratory, histopathological, and therapeutic data

Data	Response		P
	Excellent/Indeterminate n = 168 (92.8%)	Incomplete (Biochemical/Structural) n = 13 (7.2%)	
Female, n (%)[a]	146 (86.9)	12 (92.3)	0.573
Age (years)[b]	48.24 ± 13.75	51.15 ± 19.48	0.476
Lymph node dissection, n (%)[a]	68 (40.5)	7 (53.8)	0.345
Type of cancer			0.100
Papillary carcinoma, n (%)[a]	153 (91.1)	10 (76.9)	
Follicular carcinoma, n (%)[a]	15 (8.9)	3 (23.1)	
Staging (TNM)			0.230
I, n (%)[a]	110 (65.5)	7 (53.8)	
II, n (%)[a]	23 (13.7)	2 (15.4)	
III, n (%)[a]	23 (13.7)	1 (7.7)	
IV, n (%)[a]	12 (7.1)	3 (23.1)	
Risk of recurrence			**0.078**
Low, n (%)[a]	93 (55.4)	6 (46.1)	
Intermediate, n (%)[a]	50 (29.8)	2 (15,4)	
High, n (%)[a]	25 (14.9)	5 (38.5)	
Positive 1st WBS, n (%)[a]	157 (94)	13 (100)	1.00
RIT total dose (mCi)[b]	168.02 ± 86.51	383.8 ± 286.6	**<0.0001**
1st STg (ng/mL)[b]	20.4 ± 192.9	225.13 ± 585.26	**<0.001**
STg/TSH ratio[b]	0.27 ± 2.58	3.01 ± 7.8	**<0.001**
Follow-up time (months)[b]	88.16 ± 45.36	75.38 ± 39.06	0.325

1st STg: first stimulated thyroglobulin; mCi: milicuries; n: number; %: percentage; RTI: radioactive iodine ablation/therapy; TNM: American Joint Commission on Cancer (AJCC) tumor staging system; TSH: thyrotropin; WBS: whole-body scan.

[a] Frequencies and percentages; Chi-square and Fisher's exact tests.

[b] Mean ± standard deviation; Student's t-test. Statistical significance: p < 0,05.

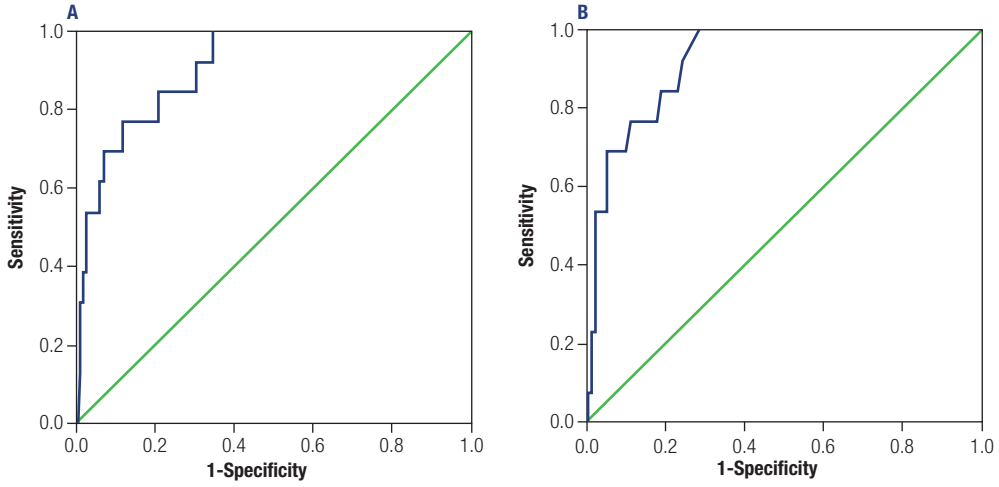

Figure 1. Receiver-operating characteristic (ROC) curves of the first stimulated thyroglobulin [A: cutoff = 5.0 ng/mL ng/mL (area under the curve: 0.907; p < 0.001)] and regarding first stimulated thyroglobulin/thyroid-stimulating hormone ratio [B: cutoff = 0.085 (area under the curve: 0.920; p < 0.001)] as predictors of incomplete response (biochemical and/or structural) in the last evaluation.

The cases with 1st TgS ≥ 5 ng/mL showed survival maintained in an excellent/indeterminate response for an average time of 15.9 months, whereas in those with 1st Tg < 5 ng/mL, this time was 140.4 months. In patients with STg/TSH ratio ≥ 0.085, this time was 15.9 months against 144.6 months in those with STg/TSH ratio < 0.085 (Figure 2).

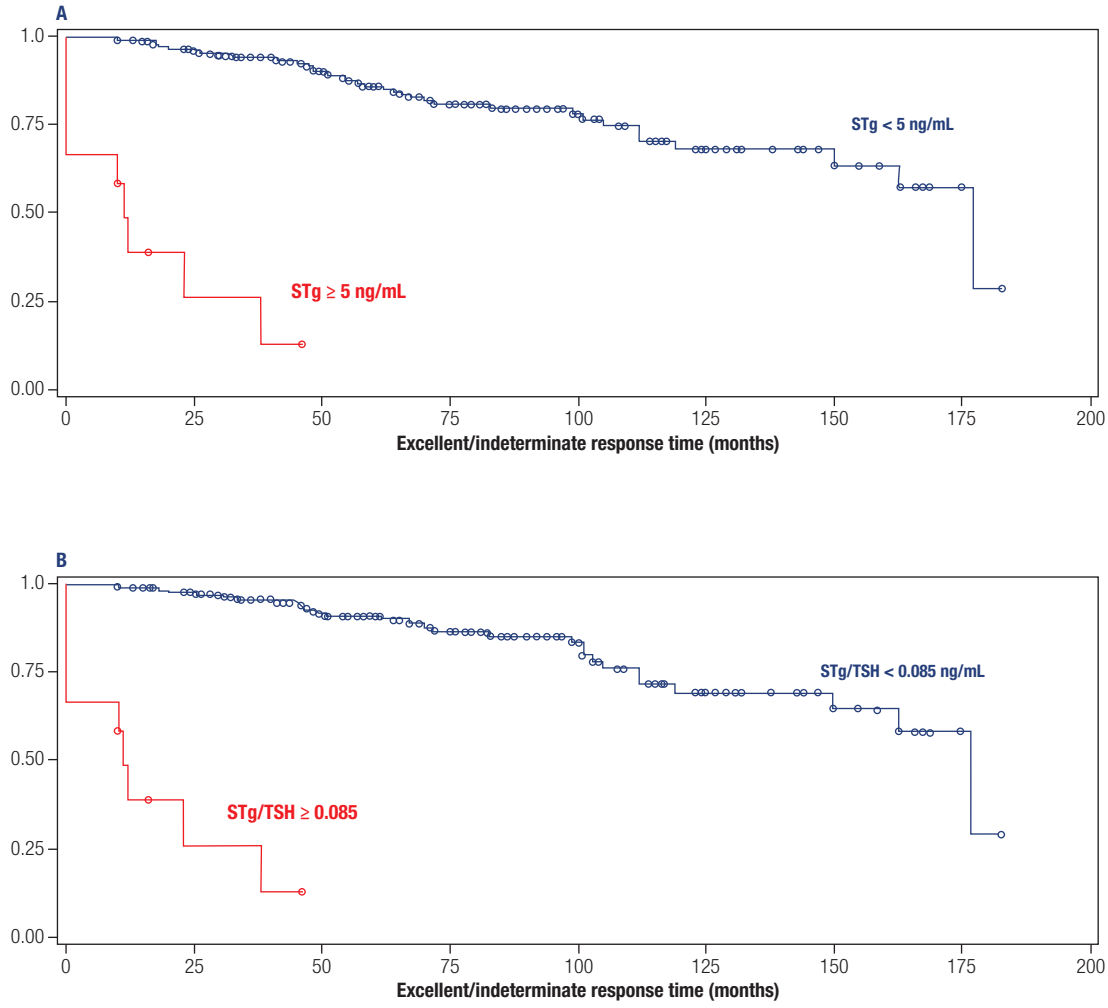

Figure 2. Kaplan-Meyer curves to assess patient survival time in excellent/indeterminate response, using the cutoff point established for the first stimulated thyroglobulin – STg as variable (A: mean survival with STg ≥ 5 ng/mL = 15.9 months and with STg < 5 ng/mL = 140.4 months; Log Rank < 0.001) and for the first stimulated thyroglobulin/thyrotrophin – STg/TSH ratio (B: mean survival with STg/TSH ≥ 0.085 = 15.9 months and with STg/TSH < 0.085 = 144.6 months; Log Rank < 0.001) in the last evaluation as predictors of outcome.

DISCUSSION

In this study we demonstrated that both 1st STg and the STg/TSH ratio, evaluated 1-3 months after TT and before 1st DTI, showed an association with long-term prognosis for DTC. Tg, and particularly STg, is already recognised as a prognosis and remission marker for the disease (17). However, as it is influenced by TSH level, it is interesting to evaluate the STg/TSH ratio, which has been associated with metastases detection and DTI effectiveness (11,15,19), but not until the completion of this study, had its potential in long-term prognosis been assessed.

In this study, after a mean follow-up time of 87 months, patients with excellent/indeterminate response at final consultation presented 1st STg lower than cases with incomplete (biochemical/structural) response. Concentrations equal or greater than 5 ng/mL were predictors of incomplete response at final consultation with elevated sensitivity, specificity, VPN, and accuracy (76.7%, 79.2%, 97.8% and 79%, respectively). Only VPP was lower, possibly influenced by the relatively small number of patients with incomplete/indeterminate response. Evaluating intermediate-risk patients, Faro and cols. observed a higher cutoff point for the marker, with STg levels> 10 ng/mL having been associated with an incomplete response (22). Perhaps the reason for the difference observed could be the time to assess the outcome, which in that study was 12 to 18 months (22). Interestingly, the 1st STg cutoff value observed in this study was very close to that seen in an earlier

study by the same service (4.41 ng/mL), and which proved to be a predictor of DTI ablative success (11). STg was also a predictor of maintenance time in response to excellent/indeterminant response during follow-up, where cases with STg < 5 ng/mL having presented a time around 8.8 times longer than those where the marker was above this level. In fact, 1st STg is recognised as a prognosis marker for DTC patients where levels below 1 ng/mL have been associated with absence of structural disease with prediction rates over 90% (7). However, the capacity of 1st STg in predicting long-term disease absence has been variable, with VPNs between 69.8% and 94.2% having been reported (7,23). As for the association between 1st STg and maintenance time in excellent response, this has generally not been a focal point in different studies thus making comparison difficult.

As already cited, despite its excellent performance, STg can be influenced by TSH level. In fact, although patients with excellent or incomplete response do not seem to significantly differ in terms of STg levels, when these are obtained with TSH values between 30-60 mU/L, higher concentrations of the pituitary hormone can cause more significant elevations in the marker, providing differentiation between these groups (18). It is true that, with the advent of recombinant TSH, this discussion could seem to be without reason. However, this is not yet widely available to the general population, and significant numbers of patients, especially those monitored in the public health service, still depend on endogenous TSH elevation for performing STg measurements. A limiting factor in this approach is that the achievement of much higher endogenous TSH levels generally implies the absence of levothyroxine treatment for long periods, which in turn can lead to a higher incidence of adverse effects and impact quality of life (24-26). In this context, correcting the marker by TSH concentration at time of evaluation, which occurs when using the STg/TSH ratio, seems more appropriate. In this study, we observed that patients with incomplete response at final evaluation had presented much higher values of this ratio soon after initial treatment. Values equal to or greater than 0.085 have even been shown as predictors of incomplete response, with high sensitivity, specificity, VPN and accuracy (76.9%, 82.6%, 97.8% and 82.2%, respectively). As with STg, only PPV was low. This cutoff value was very close to that seen in a previous study at the same service (0.093) which proved to be a predictor of DTI ablative success (11). However, it was much lower than the value reported by other authors who also evaluated ablative success (19). In our study, this ratio was also a predictor of maintenance time in excellent/indeterminate response during follow-up, with cases presenting an STg/TSH ratio < 0.085 having an around 9 times longer maintenance time than those where the ratio value was higher. No studies were found evaluating STg/TSH ratio regarding maintenance time in a given response.

This study has limitations, such as its retrospective character and the not very large sample number, particularly in relation to incomplete response cases, which could have contributed the findings obtained in analysis of association not being maintained in multivariate analysis, for instance reduced VPP seen for STg and STG/TSH ratio cutoffs. However, this study has merit in presenting the STg/TSH ratio as an additional tool for predicting long-term prognosis, considering response outcome to initial therapy in the light of the most recent recommendations for monitoring DTC. This ratio could be particularly useful in situations where the increase obtained in endogenous TSH has not been so significant.

In conclusion, our results indicate that the STg/TSH ratio has a similar performance to the 1st STg in predicting long-term response to initial therapy. Thus, these are both useful markers in evaluating prognosis for DTC patients. More studies with larger samples are necessary to investigate the real role of the STg/TSH ratio in following up these patients.

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Ten years follow up of first degree relatives of type 1 diabetes patients: presence of autoimmune biomarkers and the progression to diabetes in a retrospective cohort

[1] Departamento de Nutrologia, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brasil
[2] Faculdade de Medicina, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brasil
[3] Instituto Estadual de Diabetes e Endocrinologia Luiz Capriglione (Iede), Rio de Janeiro, RJ, Brasil

Isabella Sued Leão[1]
https://orcid.org/0000-0001-6618-4816

Débora Batista Araujo[1]
https://orcid.org/0000-0003-2969-9559

Bianca Barone[1]
https://orcid.org/0000-0002-6942-830X

Joana Rodrigues Dantas[1]
https://orcid.org/0000-0002-6088-2813

Matheus Victor de Souza Nolasco da Silva[2]
https://orcid.org/0000-0003-3281-1476

Marina Oliveira Soares[2]
https://orcid.org/0000-0003-0232-4191

Daniel Barretto Kendler[3]
https://orcid.org/0000-0003-3690-3503

Rosane Kupfer[3]
https://orcid.org/0000-0002-4073-0038

Lenita Zajdenverg[1]
https://orcid.org/0000-0002-1579-3299

Melanie Rodacki[1]
https://orcid.org/0000-0002-9007-1325

ABSTRACT

Objective: The aim of the study was to assess the autoimmunity in first degrees relatives (FDR) of patients with type 1 diabetes (T1DM) and the progression to T1DM after 10 years of follow up in the Brazilian population. **Subjects and methods:** Non-diabetic FDR of T1DM patients were interviewed and blood was drawn for autoantibodies measurement (GADA, IA-2A, IAA, ZnT8A). Serum samples were analyzed by standard radioligand binding assays performed at the Federal University of Rio de Janeiro (GADA, IAA and IA2A), and at the Skäne University Hospital, Sweden (ZnT8A). The FDR were interviewed by phone after 10 years to determine if they had developed T1DM. Descriptive statistical analysis was performed and results were described as means and standard deviation (SD). **Results:** 81 individuals were analyzed. Thirteen subjects had positive autoantibodies associated with T1DM. 10 were positive for 1 autoantibody and 3 subjects were positive for multiple autoantibodies (1 of them showed positivity for 2 autoantibodies – GADA, ZnT8A – and the other two were positive for 3 autoantibodies – GADA, IA2A, ZnT8A). The 3 subjects with multiple positive autoantibodies developed T1DM within 10 years. **Conclusions:** In Brazilian FDR of T1DM patients, the positivity for multiple autoantibodies indicate a greater chance of progression to T1DM, similar to observed in Caucasians. ZnT8A was helpful in the risk assessment for T1DM development. Arch Endocrinol Metab. 2021;65(4):436-42

Keywords
Autoimmunity; diabetes; ZnT8A; biomarker; type 1 diabetes

Correspondence to:
Isabella Sued Leão
Rua Prof. Gastão Bahiana, 619, ap. 301, Lagoa
22071-055 – Rio de Janeiro, RJ, Brasil
isabellaleao1@gmail.com

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease caused by destruction of the insulin-producing pancreatic beta cells. This process leads to insulin deficiency and dysregulation of the glycemic metabolism, which causes long-term complications and increases the morbimortality of the disease.

The development of clinically apparent T1DM is usually preceded by the appearance of autoantibodies to

islet autoantigens, such as glutamic acid decarboxylase (GADA),insulinoma-associated antigen 2/ICA512 (IA2A), insulin (IAA) and zinc transporter 8 (ZnT8A) (1-4). Several prospective studies have demonstrated that these antibodies usually appear years before the development of clinically apparent T1DM. Individuals who have developed two or more T1DM-related autoantibodies and are normoglycemic are classified as T1DM stage 1. As this disorder progresses, the patient will undergo T1DM stage 2 characterized by dysglycemia and two or more T1DM-associated autoantibodies. Finally, the individual will go through stage 3, which includes autoimmunity, dysglycemia and clinical symptoms and signs of diabetes (5).

The presence of autoimmunity does not necessarily indicate progression to T1DM stage 3. Positivity for a single autoantibody carries a relatively low risk of developing this condition, while the appearance of multiple autoantibodies (two or more) increases this risk to near certainty of progressing to diabetes (44% in 5 years, 77% in 10 years and almost 100% during the whole lifetime) (4-14). Positivity for multiple autoantibodies is used as a biomarker in risk scores for the development of T1DM, in prevention and intervention studies, along with HLA haplotypes, the first-phase insulin response, and impaired glucose tolerance (15). However, most studies in this field included only Caucasians and it is important to investigate whether the behavior of these humoral markers of diabetes follows the same pattern in multiethnic populations, when compared with Caucasians. The Brazilian population is ethnically diverse and considered one of the most heterogeneous in the world as significant admixtures within each ethnic group have been reported.

The aim of this study was to assess the autoimmunity in first degree relatives (FDRs) of patients with T1DM and the progression to T1DM after 10 years in the multiethnic Brazilian population. We hypothesized that, despite the multiethnic background of the Brazilian population, the study sample would behave like Caucasians.

SUBJECTS AND METHODS

In this retrospective cohort, subjects were recruited from May 2006 to May 2009. The sample size was defined by convenience. Data were collected in the same period. Ten years after enrollment (between 2016 and 2019), a follow-up contact by telephone was made to assess the outcome, which was the diagnosis of T1DM. Data analysis was carried out in 2019.

Subjects

Brazilian non-diabetic FDRs of patients with T1DM who were being followed up at the Diabetes Outpatient Clinic of the Federal University of Rio de Janeiro (UFRJ) and the State Institute of Diabetes and Endocrinology Luiz Capriglione (IEDE) were invited to participate in this study. The recruitment period and data collection were from May 2006 to May 2009. The initial invitation to participate in the study was made through telephone contact or at the end of the routine consultations of patients being monitored in the units mentioned above. FDRs of patients with T1DM were included.

The inclusion criteria for selection of the participants were being a child or sibling of a patient with T1DM and aged between 5 and 40 years at baseline. Only one FDR per patient was included. Exclusion criteria were confirmed diabetes mellitus or, in the case of siblings, having only one parent in common.

As for ethnicity, we chose to divide our population into Caucasians and non-Caucasians (mostly Afro-descendants), because there is a greater genetic similarity between Afro-Brazilians and Indians than with Euro-Brazilians, according to a study by Palatnik and cols. (16) This division was based on the FDR phenotype and family background.

The project was approved by the institutional ethical committee. All participants signed an informed consent form. For underage participants, the consent form was signed by a guardian.

Clinical and laboratory evaluation

Participants were interviewed and blood was drawn for autoantibodies measurement (GADA, IA-2A, IAA and ZnT8A). Serum samples were analyzed by a standard radioligand binding assay (RBA) for GAD, insulin and tyrosine phosphatase A (GADA, IAA and IA2A, respectively), which were performed at the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. The RBA to analyze the samples for the Zn-T8A comprised the three individual Zn-T8A variants (ZnT8RA, ZnT8WA and ZnT8QA) as well as the ZnT8TripleA assay, which were both developed at the Department of Clinical Sciences, Skäne University Hospital, Malmö, Sweden. Cut-off values for the positive test were set to

1.0 U/mL for GADA, IAA and IA2A, 75 U/mL for ZnT8RA and ZnT8WA, 100 U/mL for ZnT8QA and 60 U/mL for the ZnT8ATriple assay (ZnT8AQRW).

Ten years after the autoantibodies measurement, the FDRs were interviewed by phone to determine if they had developed T1DM or not. The outcome was the development of T1DM during the 10 year follow-up period. American Diabetes Association criteria were used to define diabetes. The presence of hallmark symptoms, such as polyuria, polydipsia and polyphagia and/or of weight loss before the diagnosis, ketoacidosis at diagnosis, abrupt development of diabetes and/or the need for insulin therapy were used as criteria to define T1DM, associated with the presence of autoantibodies (17).

Statistical analysis

Descriptive statistical analysis was performed and results are presented as means and standard deviation (SD).

RESULTS

Study population

A total of 81 FDRs of patients with T1DM were analyzed, (50 siblings and 31 offspring). The median age of the study group was 20 years old SD (range:5 to 46 years old). Most subjects were non-Caucasians (59.2%) and females (58%).

The population of T1DM patients also had a total of 81 patients. The mean age was 30.19 ± 11.23 years, with a predominance of non-Caucasians (55.7%) and women (61.5%). The mean age at T1DM diagnosis was 18.63 ± 11.31.

Autoantibodies measurement

Thirteen subjects (16%) had autoantibodies associated with T1DM (GADA, IAA, IA2 and/or anti-ZnT8). Ten subjects (76.9% of those) were positive for only one autoantibody, with predominance of GADA as a single autoantibody. (GADA 70%, IA2A 20%, IAA 10% in these individuals). The remaining 3 subjects (23.1% of those with positive antibodies) were positive for multiple autoantibodies.

Ten-year follow-up

Among 81 relatives screened between 2006 and 2009, 16 were lost to follow-up (19%), including 1 subject positive for a single autoantibody (IAA). Twelve of the 65 remaining FDRs (18.4%) had positive autoantibodies. The characteristics of the FDRs with positive autoantibodies are shown in Table 1.

Among the 65 FDRs who underwent follow-up, GADA was the most prevalent autoantibody, present in 9 cases. Four FDRs were positive for IA2A, 3 for ZnT8A and 1 for IAA. Of these 12 positive FDR, 1 had 2 positive autoantibodies (GADA and ZnT8A) and 2 had 3 positive autoantibodies (GADA, IA2A and ZnT8A). Three patients were negative for GADA or IAA, but were not tested for IA2A or ZnT8A. The FDR lost to follow-up with positivity for IAA tested negative for the other autoantibodies.

Three subjects with positive autoantibodies developed T1DM within 10 years. The T1DM diagnoses were established after 1, 3 and 6 years of the autoantibodies measurements. Their median age was 21.66 years old ± 5.18 at baseline and all were siblings of patients with T1DM. All these subjects had more than one positive autoantibody (2 tested positive for GADA, IA2A and ZnT8A and 1 for GADA and ZnT8A). The only individuals who developed T1DM during follow-up were those who had multiple autoantibodies (2 or more). Additionally, one subject with GADA had pre-diabetes mellitus, and had gained 10 kg in the past 10 years (BMI 38.10 kg/m^2). In this case, low GADA titers were observed (2.02 U/mL).

The results of the study are summarized in Figure 1.

DISCUSSION

In the present study, we evaluated the risk of developing T1DM in FDRs of T1DM patients based on the detection of positive autoantibodies. Our work assessed a wide age range and a multiethnic population, to identify if the findings for the Caucasians were also valid for the Brazilian population.

Of the 81 initial FDRs, 16% had one or more autoantibodies. The incidence of positive autoantibodies in our population was higher than that found in most populations studied worldwide. (7,11,14,18) and also differs from another Brazilian study (19).This may be a unique characteristic of our population, because the FDR selection criteria were similar to those of other studies in the literature (18). Among FDRs with positive autoantibodies, 75% were positive for only one autoantibody and did not develop T1DM within 10 years. Most of these individuals showed the autoantibody at low titers. The predominance of positivity for a single

Table 1. Characteristics of the FDR with positive autoantibodies

FDR	Age at autoantibody assessment	Sex	Ethnicity	Relationship	Autoantibodies	Diabetes	Age at T1DM diagnosis
HOS 1	12	Male	Non-Caucasian	Offspring (father DM)	GADA (39.73 UI/mL)	No	N/A
MSS 2	22	Male	Non-Caucasian	Offspring (mother DM)	GADA (45.31 UI/mL)	No	N/A
BLR 3	08	Female	Non-Caucasian	Offspring (father DM)	GADA (1.34 UI/mL)	No	N/A
MVP 4	33	Male	Non-Caucasian	Sibling	GADA (2.02 UI/mL)	Pre-diabetes	N/A
MSC 5	08	Male	Non-Caucasian	Sibling	IAA (1.17 UI/mL)	Lost follow up	N/A
RNS 6	28	Female	Caucasian	Sibling	GADA (1.16 UI/mL)	No	N/A
FSP 7	18	Male	Non-Caucasian	Sibling	GADA (1.4 UI/mL) IA2A (12.0 UI/mL) ZnT8AQRW (3,703 UI/mL) ZnT8AQ (2,229 UI/mL) ZnT8AR (2,810 UI/mL) ZnT8AW (2,608 UI/mL)	Yes	21
RBGF 8	20	Female	Caucasian	Sibling	IA2A (1.4 UI/mL)	No	N/A
MCMS 9	37	Female	Caucasian	Sibling	IA2A (1.3 UI/mL)	No	N/A
FSS 10	09	Female	Caucasian	Offspring (father DM)	GADA (56.73 UI/mL)	No	N/A
RTP 11	29	Male	Caucasian	Sibling	GADA (61.38 UI/mL) ZnT8AQRW (772 UI/mL) ZnT8AR (3368 UI/mL)	Yes	35
FPL 12	18	Male	Caucasian	Sibling	GADA (2.31 UI/mL)	No	N/A
JRN 13	18	Female	Caucasian	Sibling	GADA (28.02 UI/mL) IA2A (8.77 UI/mL) ZnT8AQRW (197 UI/mL) ZnT8AQ (243 UI/mL) ZnT8AR (262 UI/mL) ZnT8AW (157 UI/mL)	Yes	19

Reference values: GADA < 1.0 U/mL; IAA < 1.0 U/mL; IA2A < 1.0 U/mL; ZnT8RA < 75 U/mL; ZnT8WA < 75 U/mL; ZnT8QA < 100 U/mL; ZnT8ATriple assay (ZnT8AQRW) < 60 U/mL.
FDR: first degree relatives; N/A: not apply.

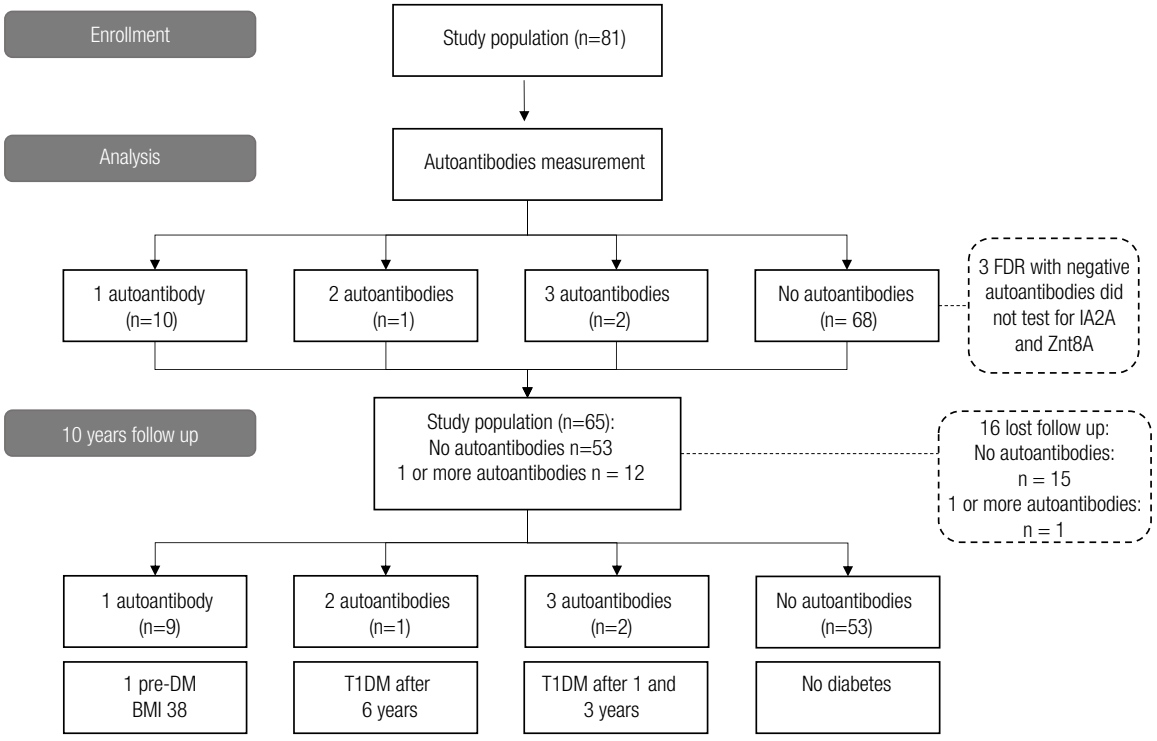

Figure 1. Flow diagram with the stages of the study – FDR: first degree relatives; DM: diabetes mellitus; T1DM: Type 1 diabetes mellitus.

autoantibody is not unique to the Brazilian population and has also been found in other populations, such as in the DPT-1, BABYDIAB, TEDDY and TrialNET study groups (6,7,11,14). The only subjects who progressed to T1DM in this period were those with two or more positive autoantibodies. This finding is in agreement with other studies performed mostly with Caucasians, which showed that the presence of a single autoantibody does not represent a high risk for the development of T1DM. On the other hand, the presence of multiple autoantibodies appears to be the hallmark of a "point of no return" in the T1DM pathogenic process, which signals the beginning of the preclinical stage of the disease (4,14,20).

ZnT8A is considered an additional diagnostic marker of T1DM that improves the overall autoantibody sensitivity. It reduced the proportion of patients with negative autoantibodies and increased the diagnostic sensitivity to over 90% for new onset cases of T1DM in Caucasians and in a Brazilian population study and to 80% in India (21-24). Furthermore, there is an independent relationship between the risk of developing T1DM and ZnT8A that favors its use in screening, especially in high-risk populations, such as FDRs of T1DM patients (23,25-27). Because most FDRs who undergo GADA, IAA and IA2A combined measurement are positive for only one of these autoantibodies, the addition of a ZnT8A test to the screening process helps to identify individuals with another positive antibody, which would represent a higher risk for T1DM (27). Interestingly, among the three subjects that developed T1DM positivity for more than one autoantibody, one would not have been detected if ZnT8A measurements had not been performed. Therefore, the ZnT8A positivity increased the number of individuals with positivity for more than one autoantibody.

Lastly, the positivity for ZnT8A conferred in the literature a greater risk of rapid progression to T1DM (15). The DAISY study showed a significantly greater risk of developing T1DM in 5 years in individuals who positive for ZnT8A when compared with those who did not exhibit positivity (8,21). In addition, the association of ZnT8A with IA2A proved to be the most sensitive combination for detecting FDRs with a high risk for rapid progression to T1DM (28,29). In this study, a fast evolution to clinical T1DM (1 and 3 years) occurred in those with ZnT8A and IA2A.

This study has some limitations. First, the sample size was small. Despite this, our results were consistent with those of other studies in the literature and the selection criteria were broad, similar to those of the DAISY (18) and TEDDY studies (11). Nevertheless, our study's population showed a higher prevalence of autoantibodies compared with other populations. This can be explained by differences in the population assessed in other studies. BABYDIAB (7) evaluated only offspring and TrialNET (14) also evaluated second and third degree relatives of T1DM patients, which can reduce the prevalence of autoantibodies in the selected population. Alves et al did not evaluate ZnT8A in a previous study with the Brazilian population (19), which may also have reduced the number of FDRs with positive autoantibodies. Moreover, our study lacked the periodic repetition of antibodies measurement and pancreatic function studies. This could be the reason for the higher prevalence of autoantibodies in this study population. Of the FDRs, 70% had low titers of a single autoantibody that might not be persistent and could have disappeared in a second evaluation. However, this publication brings important information to the field, as there is a lack of data on autoimmunity in T1DM in multiethnic populations such as the Brazilian population. A study with a larger number of participants and periodic repetition of antibodies measurement and pancreatic function studies is still required to corroborate our findings and evaluate the external validity of these results. There was a loss to follow-up in 19% patients within 10 years, including one positive for IAA. This loss to follow-up is within the expected average for long studies. One more limitation was the evaluation of progression to T1DM, which was performed only by telephone contact and not according to serum glycemia. This might resulted in underestimating the incidence of diabetes. However, diagnosis of T1DM through occasional glucose measurement is unlikely and patients were asked about specific details of T1DM at the time of diagnosis and insulin treatment, which makes the diagnosis of T1DM more reliable. Moreover three individuals were not tested for IAA and ZnT8A, which precluded us from concluding whether they were positive for multiple autoantibodies or not. Finally, it was not possible to assess the risk of developing T1DM by DPTRS or by the Genetic Risk Score due to lack of BMI and C peptide values and lack of specific genetic data (15,30).

In conclusion, this study demonstrated that positivity for only one autoantibody does not seem to confer a greater risk for developing T1DM in the Brazilian multiethnic population, whereas two or more markers indicate a greater chance of progression to the disease, which is similar to what was observed in Caucasians. ZnT8A was helpful for stratification in our population, because it was present in all three cases with multiple antibodies and its measurement allowed for the detection of multiple antibodies in one of them. Further studies are necessary to understand the role of ZnT8A in larger studies and other populations.

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Polymorphism (-499C/G) in *DDAH2* promoter may act as a protective factor for metabolic syndrome: A case-control study in Azar-Cohort population

Elnaz Faramarzi[1]
https://orcid.org/0000-0003-4128-433X

Younes Aftabi[2]
https://orcid.org/0000-0002-8692-8867

Khalil Ansarin[2]
https://orcid.org/0000-0003-1374-3316

Mohammad Hossein Somi[1]
https://orcid.org/0000-0002-6611-9958

Neda Gilani[3]
https://orcid.org/0000-0002-5399-0277

Ensiyeh Seyedrezazadeh[2]
https://orcid.org/0000-0001-5783-5218

[1] Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Department of Statistics and Epidemiology, Faculty of Health, Tabriz University of Medical Sciences, Tabriz, Iran

ABSTRACT

Objective: Globally developing metabolic syndrome (MetS) prevalence as a major health problem can be related to multiple factors of genetic and environmental. Dimethylaminohydrolase 2 (DDAH2) is the main enzyme implicated in the cardiovascular system, which regulates the nitric oxide pathway. This study investigated the association of *DDAH2* polymorphism -499C/G (rs805305) with the risk of MetS among the Azar-Cohort population. **Subjects and methods:** The occurrence of SNP rs805305 in the *DDAH2* gene was tested using the PCR-RFLP method in 332 MetS cases and 294 healthy controls. Afterward, the association of the allele and genotypes with the risk of MetS and its components were examined. **Results:** The G allele and GC genotype were significantly associated with a reduced risk of MetS ($P \leq 0.001$). Also, the dominant genetic model (GG+GC) significantly decreased the risk of MetS ($P = 0.001$), however, in sex subtypes MetS risk was significantly reduced in males before and in females after adjustment for age ($P \leq 0.02$). **Conclusion:** The -499C/G polymorphism of *DDAH2* may play a protective role and reduce MetS risk among the Azar-Cohort population. Arch Endocrinol Metab. 2021;65(4):443-9

Keywords
DDAH2; rs805305; polymorphism; metabolic syndrome; cohort study

Correspondence to:
Ensiyeh Seyedrezazadeh
Tuberculosis and Lung Disease Research Center
Pashmineh Research Complex, Daneshgah Street, Tabriz, Iran
E-mail: e.s.rezazadeh@tbzmed.ac.ir

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INTRODUCTION

Metabolic syndrome (MetS) is a bunch of various metabolic disorders, comprise obesity, insulin resistance, glucose metabolism faulty, and hypertension (1). Increasing risk of some chronic diseases including cardiovascular diseases, cancer, and diabetic mellitus attributed to metabolic syndrome as a new silent killer (2). MetS have become a noteworthy general medical issue worldwide and its prevalence is also dependent on defined criteria and a variety of regions. For instance, it is estimated its prevalence is 44.1% in Jordan, 28.9% in Turkey, and 30.4% in Iran (3-5). Despite the global prevalence increment of MetS, its etiology is not entirely recognized. Multiple risk factors, including obesity, insulin resistance, low-grade inflammation, oxidative stress, environmental, and genetic factors, are attributed to play major roles in MetS development (6,7).

Nowadays, the roles of genetic factors are more considered. In this sense, the reduction level of nitric oxide (NO) as a key bioactive molecule produced by nitric oxide synthase is critically involved in pathophysiological events of MetS. Dysregulation of the NO pathway is mainly attributed to increased levels of asymmetric dimethylarginine (ADMA). ADMA is originated from the proteolysis of various proteins with methylated

arginine residues. It is an endogenous inhibitor of nitric oxide synthase (NOS) which is complicated in MetS pathogenesis (8). Moreover, ADMA degrades via the action of dimethylarginine dimethylaminohydrolase (DDAH) enzymes, that two isoforms of them have been determined: DDAH1 is believed to be associated with neuronal NOS (nNOS) and DDAH2 is thought to be related with tissue endothelial NOS (eNOS) expression (9). These enzymes are encoded on chromosomes 1p22 and 6p21.3 (10). As reported, with high oxidative stress conditions in MetS, the activity of *DDAH2* reduced (11). More than 70% of the ADMA level is metabolized by DDAH 2, hence reduction of DDAH2 activity induces ADMA level elevation, which in turn decreases NO signaling and according to that increments of systemic resistance of vascular and also systemic and pulmonary blood pressure are occurred (12). Recent studies have established DDAH2 has the main contribution to NO activity in endothelial cells. Consequently, *DDAH2* gene silencing decreases about 40% endothelial relaxation (13). Confirming recent studies the prevalence of the dysfunctional *DDAH2* gene variants could make enzyme activity variants that affect the risk of MetS related diseases. Seo and cols. reported that SNP rs2272592 of *DDAH2* in contrast to SNP rs805304 is in the relation to type 2 diabetes (11), while the rs805304 C allele was connected to the risk of myocardial infarction and obesity decrement (14). As reported by Xuan and cols., patients with coronary artery disease exhibited a significant correlation of the *DDAH2* genetic polymorphism (-499C/G, rs805305) with plasma ADMA (15).

Taking into account the crucial role of hypertension and diabetes in MetS, as well as inconclusive results about the association between MetS and *DDAH2*, it appeared that genetic polymorphism in *DDAH2* may contain biomarkers for the correlation of MetS. Therefore, this study's purpose was mainly to assess the association of the DDAH2 polymorphism with the risk of MetS among the Azar cohort population.

SUBJECTS AND METHODS

Subjects

In this case-control study, 626 adult subjects, including 332 with MetS and 294 healthy controls drawn from the Azar Cohort study, the large Iranian prospective epidemiological research study (Persian Cohort), were included (16). More details of this study were reported previously (17). Participants with at least three items of MetS criteria as cases or healthy volunteers as controls and aged 35-50 years old were included. Individuals who smoked or used hookah, drug abuse (addiction), and drank alcohol were excluded. Participants have been notified of the purpose of the study and then written their consent. The Ethics Committee of the Tabriz University of Medical Sciences (confirmation code: IR.TBZMED.REC.1399.257) was confirmed the present research. Demographic characteristics were also collected using a questionnaire.

Anthropometric factors, MetS components, and blood sampling

Using NIH guidelines, anthropometric factors, including weight, heights, and waist circumference, were measured, and using the formula kg/m^2 the body mass index (BMI) was determined. Blood pressure (BP) was measured twice a day with 2-minute intervals in each arm in a sitting position after a 10-minute rest period using a mercury sphygmomanometer (Rudolf Richter; DE-72417; Germany). The averages of these two measurements were used as the daily systolic and diastolic blood pressure measurements. Following 12 hours of overnight fasting blood samples were collected. The enzymatic methods were used for measuring serum levels of fasting blood sugar (FBS), triglyceride (TG), total cholesterol (TC), and high-density lipoprotein (HDL) (16). Friedewald's formula was used for calculating Low-density lipoprotein (LDL) (18). Besides, 100 µL of the blood sample was frizzed at -80 °C for DNA extraction by using a blood DNA extraction kit (DNA Biotech, Iran).

Metabolic syndrome definition

This study used the National Cholesterol Education Program Adult Treatment Panel III report criteria (ATP III) for selecting MetS participants (19). Participants with at least three of the following criteria were defined as MetS cases: waist circumference ≥ 102 cm for men and ≥ 88 cm for women, TG ≥ 150 mg/dL or drug treatment for elevated triglycerides as an alternate indicator, and HDL-C values of < 40 mg/dL for men and < 50 mg/dL for women. Hypertension was defined as systolic blood pressure ≥ 130 mmHg and/or diastolic ≥ 85 mmHg or the use of antihypertensive medication.

Elevated fasting glucose was considered to be ≥ 100 mg/dL or the use of glucose-lowering medication.

PCR-RFLP and SNP genotyping

The genotyping of SNP rs805305 was performed by the PCR-RFLP method using specific forward, 5′-CCTTCTCGTTCGGGTATTCAG-3′; and reverse, 5′-TCCAGACCTTCCGCTCCT-3′ primers and restriction enzyme SmaI. Briefly, to amplify the fragments, we used 20 µL of PCR reaction mix including 10 µL Master Mix RED (5200300-1250: Ampliqon, Denmark), 0.5 µL (10 pmol/µL) of each forward and reverse primers (Bioneer, Takapouzist, Iran), 2 µL of the extracted DNA (50 ngr/µL) as a template and 7.5 µL deionized water. Thermal cycling was performed in a Primus 96 advanced thermal cycler (PEQLAB, Erlangen, Germany) under the following conditions: an initial hot start at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 60 sec, annealing at 58 °C for 45 sec, and extension at 72 °C for 40 sec, and final elongation at 72 °C for 5 min. The amplified PCR product (4 µL) was digested with 2 µL SmaI (BioLabs; UK) in 2 µL 10X buffer with deionized water to a final volume of 20 µL in a 30 °C water bath for 4 h. After digestion, the treated mixture was electrophoresed on 2% agarose gel, and alleles (G: 341 bp; C: 254 and 87 bp) were visualized by dual intensity transilluminator (UVP, Upland, USA). Quality control genotyping was done using blind test assessments. Moreover, to verify the reproducibility of the results a 5% random sample of participants was genotyped twice by different operators (20).

Statistical analysis

Data are presented as mean ± SD for numeric variables and frequency (percentage) for qualitative ones. Between groups, an independent t-test was used for quantitative variables comparison and chi-square tests for qualitative one's comparison. The $\chi 2$ test was applied for assessing Deviation from the Hardy-Weinberg equilibrium. Logistic regression analyses were used by adjusting for age and sex and displayed as odds ratios (ORs) and 95% confidence intervals (CIs). A two-tailed $P_{value} \leq 0.05$ was considered significant. Statistical analyses were performed by using SPSS software version 17 (Chicago, IL, USA).

RESULTS

Baseline characteristics of participants were stratified by case group and control group, and these are displayed in Table 1. Of 332 participants with MetS, 42.5% were men, and 57.5%were women. The control group (294 participants) included 40.8% men and 59.2% women. Table 1 displayed the significant differences between the case and control groups regarding anthropometric parameters and serum levels of MetS components and also between males and females ($P < 0.001$). Additionally, significant differences were seen in the serum levels of LDL between groups ($P \leq 0.01$), (Table 1).

Allele distribution and genotype frequency and association of *DDAH2* with MetS are shown in Table 2. Quality control test by repeating the genotyping for 5% randomly selected DNA samples did not disagree with the outcome of original genotyping. The higher distribution of G allele and CG genotype in the control groups showed a significant association between this genotype and a lower risk of MetS even after adjusting for age and gender ($P \leq 0.001$). The genotypes frequencies of the total, male, and female samples in the control group had non-significant deviation ($P > 0.05$) from Hardy-Weinberg Equilibrium (HWE) (Table 3). Moreover, the G dominant genetic model (GG+GC) as an SNP rs805305 subtype, showed a significantly reduced risk of MetS ($P = 0.001$) (Table 2). Similarly, an increased percentage of CG genotype had a significantly lower risk of MetS in both males and females (Table 2). Although the G dominant genotype (GG+GC) significantly reduced the MetS risk in males, before adjusting for age, and in females after adjusting for age, the C dominant model (GC+CC) showed non-significant effects.

DISCUSSION

Further investigation is required around the effects of variant genotypes on the risk of metabolic syndrome as a public health problem and related diseases. In this case, the apparent mechanism of the polymorphism function of *DDAH2* in MetS is mainly unclear. Therefore, the current study evaluated the role of -499C/G, rs805305 polymorphism in the *DDAH2* gene on MetS. As far as our knowledge is concerned, this is the first study that examines the relationship between 499C/G, rs805305 polymorphism of the *DDAH2* gene, and the risk of MetS. As would be expected, the present

Table 1. MetS components and anthropometric factors of the participants

Parameters	MetS Patients (n = 332)	Controls (n = 294)	Pvalue
Total	**mean ± SD**	**mean ± SD**	
Age (years)	43.05 ± 4.44	41.70 ± 4.50	<0.001
Male n(%)	141 (42.5)	120 (40.8)	
Female n(%)	191 (57.5)	174 (59.2)	
Weight (kg)	85.11 ± 13.37	74.07 ± 13.16	<0.001
BMI (kg/m^2)	32.01 ± 4.18	27.83 ± 4.93	<0.001
Waist circumference (cm)	99.33 ± 9.22	87.93 ± 10.19	<0.001
SBP (mmHg)	119.45 ± 14.49	111.95 ± 12.83	<0.001
DBP (mmHg)	79.22 ± 8.89	73.11 ± 8.59	<0.001
Glucose (mg/dL)	100.03 ± 14.97	91.35 ± 9.49	<0.001
Total cholesterol (mg/dL)	204.92 ± 43.53	187.48 ± 33.72	<0.001
LDL (mg/dL)	121.04 ± 34.45	114.59 ± 32.13	0.01
HDL (mg/dL)	40.27 ± 9.73	49.68 ± 11.59	<0.001
Triglycerides (mg/dL)	215.3 ± 120.88	120.23 ± 41.57	<0.001
Male	**N = 141**	**N = 120**	
Age (years)	42.60 ± 4.40	42.29 ± 4.45	0.65
Weight (kg)	91.05 ± 13.79	77.21 ± 11.44	<0.001
BMI (kg/m^2)	31.00 ± 3.84	25.96 ± 3.54	<0.001
Waist circumference (cm)	100.21 ± 9.22	88.10 ± 9.89	<0.001
SBP (mmHg)	121.98 ± 14.13	115.77 ± 14.09	<0.001
DBP (mmHg)	82.05 ± 9.88	75.57 ± 8.99	<0.001
Glucose (mg/dL)	103.02 ± 18.74	96.01 ± 8.96	<0.001
Total cholesterol (mg/dL)	205.54 ± 47.39	191.75 ± 37.58	0.01
LDL (mg/dL)	120.10 ± 42.86	119.16 ± 34.50	0.84
HDL (mg/dL)	36.09 ± 7.82	46.61 ± 10.86	<0.001
Triglycerides (mg/dL)	250.20 ± 153.94	129.83 ± 55.23	<0.001
Female	**N = 191**	**N = 174**	
Age (years)	43.26 ± 4.46	41.43 ± 4.52	<0.001
Weight (kg)	80.75 ± 11.30	71.90 ± 13.85	<0.001
BMI (kg/m^2)	32.72 ± 4.30	29.11 ± 5.34	<0.001
Waist circumference (cm)	97.99 ± 9.01	87.90 ± 10.56	<0.001
SBP (mmHg)	117.61 ± 14.50	109.31 ± 11.18	<0.001
DBP (mmHg)	77.17 ± 7.44	71.42 ± 7.90	<0.001
Glucose (mg/dL)	97.79 ± 11.04	88.14 ± 8.48	<0.001
Total cholesterol (mg/dL)	204.67 ± 40.56	185.97 ± 34.06	<0.001
LDL (mg/dL)	123.27 ± 33.69	111.44 ± 30.09	0.001
HDL (mg/dL)	43.06 ± 7.82	51.79 ± 11.64	<0.001
Triglycerides (mg/dL)	191.60 ± 82.05	113.62 ± 26.86	<0.001

BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure;
DBP: diastolic blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein. *$P_{value} < 0.05$.

study demonstrated substantial differences in MetS components between MetS cases and controls with and without gender classification. Moreover, the finding indicated that carriers of G allele, GC genotype, and G dominant genetic model, (GG+GC) compared to CC, manifested a lower risk of MetS compared to individuals conveying genotype GG, and C recessive genetic model GG vs (GC+CC). The main concern regarding

Table 2. Association of DDAH2 c. -449 C/G SNP with MetS

Genotype/Allele	MetS Patients N (%)	Controls N (%)	Unadjusted Odds Ratio (95% CI)	Pvalue	*Adjusted Odds Ratio (95% CI)	Pvalue
Total (N = 626)	(N = 332)	(N = 294)				
CC	130 (39.2)	80 (27.4)	Reference			
CG	133 (40.1)	158 (54.1)	0.51 (0.36-0.74)	<0.0001	0.50 (0.34-0.74)	0.001
GG	69 (20.8)	54 (18.5)	0.78 (0.5-1.23)	0.29	0.76 (0.45-1.29)	0.32
C	393 (59.2)	313 (53.6)	Reference			
G	271 (40.8)	271 (46.4)	0.74 (0.63-0.99)	0.04	0.67 (0.54-0.86)	0.002
(GG+GC) vs. CC	202 (60.8) vs. 130 (39.2)	212 (72.6) vs. 80 (27.4)	0.58 (0.41-0.82)	0.002	0.56 (0.39-0.81)	0.002
HWE	$X^2 = 9.69$; $P < 0.005$	$X^2 = 2.41$; $P > 0.05$				
Male (n = 259)	(N = 141)	(N = 118)				
CC	45 (31.9)	23 (19.5)	Ref.			
CG	61 (43.3)	68 (57.6)	0.45 (0.24-0.84)	0.01	0.49 (0.24-0.98)	0.04
GG	35 (24.8)	27 (22.9)	0.66 (0.32-1.34)	0.25	0.73 (0.28-1.87)	0.52
C	151 (53.5)	109 (46.4)	Ref.			
G	131 (46.5)	127 (53.8)	0.74 (0.52-1.05)	0.09	0.89 (0.60-1.33)	0.58
(GG+GC) vs. CC	96 (68.1) vs. 45 (31.9)	95 (80.5) vs. 23 (19.5)	0.51 (0.29-0.92)	0.02	0.54 (0.28-1.04)	0.06
HWE	X2 = 2.4; P > 0.05	X2 = 2.79; P > 0.05				
Female (n = 365)	(N = 191)	(N = 174)				
CC	85 (44.5)	57 (32.8)	Ref.			
CG	72 (37.7)	90 (51.7)	0.53 (0.34-0.84)	0.008	0.51 (0.32-0.82)	0.006
GG	34 (17.8)	27 (15.5)	0.84 (0.46-1.54)	0.58	0.78 (0.41-1.49)	0.46
C	242 (63.4)	204 (58.6)	Ref.			
G	140 (36.6)	144 (41.4)	0.82 (0.6-1.10)	0.19	0.55 (0.40-0.77)	<0.0001
(GG+GC) vs. CC	106 (55.5) vs. 85 (44.5)	117 (67.2) vs. 57 (32.28)	0.60 (0.39-0.93)	0.22	0.57 (0.36-0.89)	0.01
HWE	X2 = 6.76; P < 0.01	X2 = 0.76; P > 0.05				

*Adjusted odds ratio (95% CI) for age and sex in total sample of study; adjusted for age in both gender. *P < 0.05.

Table 3. HWE estimation for control groups

	Total		Male		Female	
	*Observed #	Expected #	*Observed #	Expected #	*Observed #	Expected #
CC	80	86.6	23	27.5	57	59.8
CG	158	144.8	68	58.9	90	84.4
GG	54	60.6	27	31.5	27	29.8
Chi-squared value	2.410		2.794		0.762	
P value	0.121		0.095		0.383	

the finding of this study is the fact that the G allele is significantly associated with the decreased odds ratios of MetS in total and female samples. Also, in the male subpopulation, the odds ratio of the G allele was lower than 1 but insignificant. However, although the odds ratios of GG genotype in total, male, and female samples were decreasing (0.76, 0.73, and 0.78 respectively) their associations were non-significant. It is probably was due to sample sizes. Pérez-Hernández and cols. found that the rs805304 C allele of *DDAH2* plays a protective role in patients with myocardial infarction. Moreover, they opined that this allele of the rs805304 polymorphism was related to the risk of obesity reduction as well (14). On the other hand, a non-significant association was shown

between the CC genotype of SNP rs805304 (-1151 C/A) and its dominant genotype model (AG+GG) and diabetes and hypertension (11). Maas and cols. declared that the polymorphisms of -1151 A/C and -449 G/C located on the *DDAH2* promoter region were associated with the prevalence of hypertension enhancement (21). It is acknowledged that the G allele of the *DDAH2* gene – 499 C/G polymorphism is a major risk factor for male Egyptian CAD patients with 35-50-year-old (22). Although a significant relation was exhibited between the higher plasma level of ADMA and – 499 C/G rs 805305 polymorphism, no relation was reported between the *DDAH* 2 polymorphisms and the risk of CAD (15,23). This inconsistency may be ascribed to sample size insufficiency, or different susceptibility, ethnic diversity, or environmental factors, and their impact on genes. Evidence suggests that DDAH2 via some mechanisms has revealed a protective role on MetS.

Previous studies indicated the common effects of DDAH2 on hypertension, diabetes, CVD is attributed to ADMA. It has been reported that high levels of ADMA are associated with hypertension, type2 diabetes, and insulin resistance (24,25). DDAH2 is the main regulator of ADMA levels, a decreased level of *DDAH2* expression that can lead to an increase in ADMA concentration (15). High serum ADMA levels through various mechanisms resulted in an increased risk of metabolic syndrome components. In this way, Chen and cols. alleged that dose-dependent incubation of oxidized low-density lipoprotein (oxLDL) decreased DDAH2 protein expression. In contrast, the increment level of ADMA was induced by oxLDL. They concluded that the DDAH2/ADMA system during the transformation of macrophage foam cells may regulate lipid metabolism; additionally, its protective role may observe in deregulated lipid metabolism of foam cells of macrophages (26).

The strength of the current study is a sample size selected from the cohort population of the same ethnicity. Moreover, this is the first study that examined the relationship between 499C/G, rs805305 polymorphism in the *DDAH2* gene, and MetS. Also, we recently reported the risk-increasing role of *NOS3*-c.894G>T in MetS in the Azar-cohort population, which affects another component of the NO pathway, the eNOS enzyme (27). Despite these strong points, this study has some limitations. First, the cross-sectional design of the research was allowing no causal interferences. Second, the relation of other polymorphisms of MetS was not examined. Besides, for the reason the ADMA levels and nitric oxide activity were not measured, reporting the mechanisms that underlying the relationship between *DDAH2* gene variation and MetS should be inferred with caution. Finally, the small sample size was another limitation of this study. The effect of this limitation was seen especially when we found that although there was a significant association with the G allele, the GG genotype was not associated significantly (Table 2). However, by testing analysis for a hypothetical case and control groups with doubled size (Controls: CC: 160, CG: 300, GG: 316; Cases: CC: 260, CG: 266, GG: 138) we can see that the ORs are lower than 1 for all comparisons as we reported, however, the GG genotype also shows the significant effect ($P < 0.0001$) on decreased OR (data not shown).

In conclusion, according to these study findings, the G allele and CG genotype of *DDAH2* rs805305 polymorphism and its G dominant genotype model is significantly associated with a lower risk of MetS. Considering gene-gene interactions, further research is needed to elucidate the implication of other gene variants of *DDAH2* on MetS criteria to provide more accurate results in diverse ethnic populations. Considering that SNPs association studies are prone to spurious associations by chance, therefore it is suggested that larger samples size could be evaluated and replication in other groups could be tried.

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Perinatal effects of maternal FT3/FT4 ratio on gestational transient thyrotoxicosis

[1] Department of Endocrinology and Metabolism, University of Mustafa Kemal, Hatay, Turkey
[2] Department of Obstetrics and Gynecology, University of Mustafa Kemal, Hatay, Turkey
[3] Department of Medical Informatics and Biostatistics, University of Mustafa Kemal, Hatay, Turkey

Eren Gürkan[1]
https://orcid.org/0000-0002-3118-4549

Kenan Dolapçıoğlu[2]
https://orcid.org/0000-0002-2296-9037

Emre Dirican[3]
https://orcid.org/0000-0003-3550-1326

ABSTRACT

Objective: The effects of maternal thyroid hormone levels on the course of pregnancy and birth weight have attracted interest. The aim of the present study was to consider FT3 and FT3/FT4 ratio in the evaluation of the effects of maternal thyroid functions in gestational transient thyrotoxicosis (GTT). **Materials and methods:** This case-control study included 45 patients with GTT and 45 healthy pregnant women. Maternal history before pregnancy, thyroid function tests, thyroid autoantibodies, and thyroid ultrasonography results in 6th to 10th weeks of pregnancy were used in the differential diagnosis of GTT. In both groups, the effects of FT3, FT4 and FT3/FT4 ratios on gestational age and birth weight were evaluated. **Results:** There was no significant difference in the gestational age between the GTT and control groups (39,3±1,0 weeks and 39,2±1,2 weeks, respectively). Birth weights were similar in both groups (3205,2±4899 g and 3196,6±309,3 g, respectively). When maternal weight was adjusted, a positive correlation was observed between maternal FT3/FT4 ratio and birth weight (r=0,317, p=0,017). Additionally there was a positive correlation between the gestational age and the birth weight in the control group (ρ=0,726, p=0,001). **Conclusion:** GTT had no significant effect on the gestational age and the birth weight. On the other hand an increase in the maternal FT3/FT4 ratio had a positive effect on the birth weight in the patient with GTT. Maternal characteristics (age, weight, BMI) and FT3/FT4 ratio should be taken into consideration in future impact assessment studies on this issue. Arch Endocrinol Metab. 2021;65(4):450-4

Keywords
Gestational transient thyrotoxicosis; maternal weight; birth weight; gestational age; thyroid function tests

Correspondence to:
Eren Gürkan
Hatay MKÜ Tayfur Ata Sökmen Tıp Fak Endokrinoloji ve Met. Hast. BD
Alahan Mah, Antakya, Hatay
erengurkan@ttmail.com

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INTRODUCTION

Mild hyperthyroidism may occur in early stages of pregnancy due to an effect of increased human chorionic gonadotropin hormone. This condition is defined as gestational transient thyrotoxicosis and it is the most common cause of thyrotoxicosis in the first trimester of pregnancy. The incidence of GTT during pregnancy varies between 1-3%. While serum thyroid-stimulating hormone (TSH) levels are suppressed in GTT, FT4 and FT3 levels are normal or increase. The most important serological characteristic of patients with overt GTT is high FT3 level (1-4).

Gestational transient thyrotoxicosis is distinguished from Graves' disease by absence of hyperthyroidism before pregnancy, absence of ophthalmopathy and goiter on physical examination, lack of TSH receptor antibody in serum, and normalization of thyroid functions in the second trimester (4). There is no indication for using antithyroid drugs in GTT (5).

Presence of hyperthyroidism during pregnancy is associated with low birth weight of a newborn. Compared to patients without hyperthyroidism during pregnancy, the rate of low birth weight increases by 2.3 times in patients with controlled hyperthyroidism and 9.2 times in those with uncontrolled course. This situation can be often associated with premature birth (6). In the study by Lazarus and Kaklamanou (7), higher rate of poor pregnancy outcomes were observed in the pregnant women with Graves' disease compared to those with GTT.

In pregnant women with hyperthyroidism who achieve euthyroid state with treatment, the risk of reduced fetal growth, premature birth, and low birth weight increase. In addition, pregnancy-induced

hypertension can also be at a high rate in these women (8). Maternal FT4 levels in the early period of pregnancy and its perinatal effects have also been investigated (9). The aim of the present study was to consider FT3 and FT3/FT4 ratio in the evaluation of the effects of maternal thyroid functions in GTT.

MATERIALS AND METHODS

The present study was designed as a retrospective case-control study in which data of patients were obtained through the hospital records. The present study was approved by the Ethics Committee of Hatay Mustafa Kemal University (date: 27.02.2020 and approval no: 03).

Data of 45 patients who applied to the endocrinology outpatient clinic of Hatay Mustafa Kemal University Faculty of Medicine from September 2015 through December 2016 and who were diagnosed with thyrotoxicosis via thyroid function tests (TFTs) between the 6th and 10th weeks of their pregnancies were analyzed. Absence of a known thyroid disease, serum TSH levels being <0.1 IU/mL, normal or high serum FT4 and/or FT3 levels, and negative thyroid auto-antibodies (anti-TPO, anti-Tg, and TSH receptor antibody) were accepted as the basic criteria for the diagnosis of GTT (10). Patients who previously received radiotherapy, had thyroid disease before pregnancy, and had a history of gestational trophoblastic disease were excluded. Patients losing 5% of their body weight during pregnancy or having ketonuria and frequent vomiting were considered to have hyperemesis gravidarum and they were also excluded from the study. Control group was composed of 45 pregnant women who applied to the gynecology and obstetrics outpatient clinic within the same dates and who were between the 6th and 10th weeks of their pregnancies. The women in the control group were followed-up by the same physician, had normal TFT (FT3, FT4, and TSH) values and normal thyroid ultrasonography findings, had negative thyroid autoantibodies, and had no additional systematic diseases.

On the patients' monitoring system, the patients with GTT had monthly follow-up records until their TFT values were normalized in 2nd trimester. The control group had at least one normal TFT result in each trimester until the end of their pregnancy periods. Data relating to the pregnancy and perinatal outcomes were obtained from both the maternity service records and the pregnant follow-up system in our city. Besides, missing data in the anamnesis of the patients were obtained by phone calls to the patients.

In our clinic, blood samples were routinely collected in the morning after overnight fasting. For TFT and thyroid autoantibodies, chemiluminescent immunoassay technique (Cobas E6000, Roche Diagnostic, Germany) was used. Ultrasonographic measurements were performed by an experienced radiologist using the MyLab ClassC ultrasonography device (Esaote SpA, Italy) with a 14-MHz linear prob.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). In descriptive statistics, numerical variables were expressed as mean, standard deviation, median, minimum, and maximum and categorical variables were expressed as number and percentage. For the normality analysis of variables, the Shapiro-Wilk test was used. In the analyses, student-t test, Mann-Whitney U test, Chi-square test, Pearson correlation, Spearman's rank correlation, and Partial correlation were used. While drawing scatter graphics in partial correlation, unstandardized residual was calculated and drawn over linear regression. A p value <0.05 was accepted as a statistical significance limit within 95% confidence interval.

RESULTS

There were no significant differences in maternal characteristics (age, weight, and BMI) between the GTT and control groups (Table 1). The median number of births was slightly higher in the GTT group than in the control group, but the difference was borderline significant (2.5 vs. 2, p=0.053). As expected, FT4, FT3, and TSH values were statistically significantly higher in the GTT group as compared to controls (p=0.001 for all). There were no significant differences between the GTT and control groups according to the gestational ages during birth, birth weights, and sex of the newborns (p=0.882, p=0.918, and p=0.527, respectively) (Table 1).

There was no relationship between the weight and birth weight (r=0.019, p=0.908) in the GTT group. When the effects of maternal thyroid hormones were evaluated, a positive correlation was found between the maternal weight and FT3/FT4 ratio (r=0.302, p=0.049) in this group. A positive correlation was

observed between the birth weight and FT3/FT4 ratio in the GTT group (r=0.317, p=0.017) (Table 2 and Figure 1). However, no such correlation was obtained in the control group (Table 2 and Figure 2). On the other hand, when weight, as a confounding factor, was adjusted in the partial correlation analysis, there was no significant correlation between the FT3/FT4 ratio and the gestational week in both study groups (r=0.146 and p=0.376 in the GTT group and r=0.050 and p=0.752 in the control group).

According to the Spearman's rank correlation analysis results in the study groups, the increase in the gestational age in the GTT group had no significant effect on the birth weight (p=0.194, p=0.140). However, such correlation was found significant in the control group (p=0.726, p=0.001).

Table 1. Characteristic data of our study groups

Characteristics	GTT group		Control group		p
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
Age (year)	30.1 ± 6.4	30 (18-44)	32.2 ± 5.7	32 (20-49)	0.075[a]
Weight (kg)	66.5 ± 13.1	68 (50-98)	64.8 ± 9.5	63.5 (49-87)	0.718[b]
BMI (kg/m^2)	25.4 ± 4.7	24.9 (18.8-37.5)	24.8 ± 4.5	23.8 (19.5-39.7)	0.505[b]
Number of births	2.7 ± 1.6	2.5 (1-7)	1.9 ± 0.8	2 (1-4)	0.053[b]
FT4 (ng/dL)	1.9 ± 0.7	1.75 (0.9-3.8)	1.2 ± 0.2	1.2 (0.5-1.7)	0.001[b]
FT3 (pg/mL)	5.0 ± 1.4	4.9 (2.7-9.0)	3.2 ± 1.1	3.2 (1.2-5.5)	0.001[b]
FT3/FT4	2.8 ± 0.5	2.7 (1.5–3.8)	2.8 ± 1.1	2.5 (1.1–6.0)	0.215[b]
TSH (IU/mL)	0.02 ± 0.02	0.01 (0.00-0.01)	1.72 ± 0.61	1.70 (0.30-3.26)	0.001[b]
Gestational age during birth (wk)	39.3 ± 1.0	40 (37-40)	39.2 ± 1.2	40 (37-41)	0.882[b]
Birth weight (g)	3205 ± 490	3245 (1480-4300)	3197 ± 309	3240 (2640-3930)	0.918[a]
Newborn sex (n (%))					
Girl	25 (53.2)		22 (46.8)		0.527[c]
Boy	20 (46.5)		23 (53.5)		

[a]Student-t Test; [b]Mann-Whitney U Test; [c]Chi-Square Test, wk: week.
BMI: body mass index; GTT: gestational transient thyrotoxicosis; SD: standard deviation; TSH: thyroid-stimulating hormone.

Table 2. Correlation analysis between maternal thyroid hormones and birth weight by adjusting weight as a confounding factor in the study groups

	Confounding factor	Parameters	Statistics	Birth weight
GTT group	Weight	FT4	r	-0.093
			p	0.577
		FT3	r	0.131
			p	0.435
		FT3/FT4	r	0.317
			p	0.017
Control group		FT4	r	-0.012
			p	0.941
		FT3	r	-0.066
			p	0.677
		FT3/FT4	r	-0.067
			p	0.674

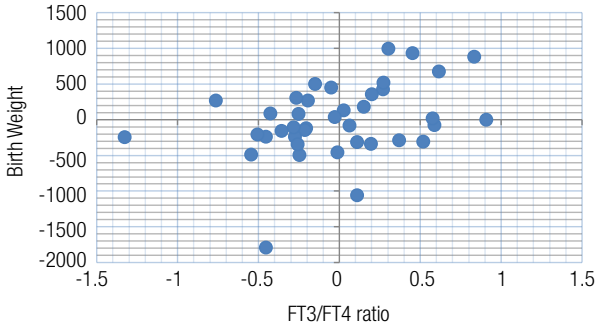

Figure 1. Partial correlation analysis between the birth weight and FT3/FT4 ratio in the gestational transient thyrotoxicosis group (weight control variable). Values in the graphic are calculated as per non-standardized residues.

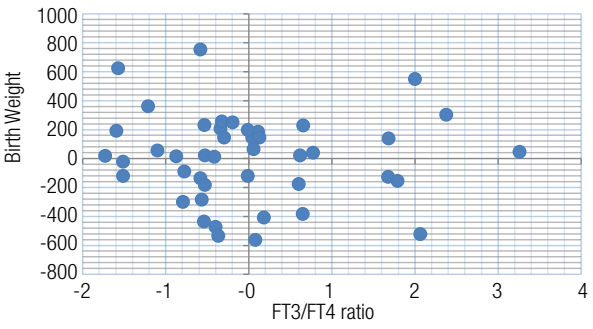

Figure 2. Partial correlation analysis between the birth weight and FT3/FT4 ratio in the control group (weight control variable). Values in the graphic are calculated as per non-standardized residues.

DISCUSSION

In the current case-control study including the patients with GTT and the pregnant women having normal thyroid functions, the mean gestational age of the groups during birth were compared and no significant difference was obtained (39.3±1.0 weeks in the GTT group and 39.2±1.2 weeks in the control group, p=0.882). Similarly Kinomoto-Kondo and cols. (11) evaluated the effects of GTT on pregnancy outcomes in their case-control study. While they reported no significant difference between the GTT patients and controls regarding low birth weight, preeclampsia, pregnancy-induced hypertension, ablation placenta, and prematurity, they found the gestational age at birth being significantly lower in the GTT group (11). In the present study, no negative effects of GTT on pregnancy-related results (preeclampsia, ablation placenta, prematurity, gestational diabetes, etc.) were observed too. However, the discrepancy observed in the results of gestational age at birth between the present study and the above-mentioned study could be attributed to the difference in the number of patients included in the studies.

According to the results of the correlation analysis between the birth weight and gestational age in the present study, the increase in the gestational age in the GTT group did not affect the birth weight (p=0.194 and p=0.140). However, a positive significant correlation was observed between the birth weight and gestational age in the control group (p=0.726 and p=0.001). This correlation observed in the control group may help to explain the difference in birth weights between our study and other studies. In our study, gestational age in patients with GTT was >37 weeks and this was compatible with the literature (11).

In the present study, as expected, maternal FT4 and FT3 values were significantly higher and maternal TSH values were significantly lower in the GTT group as compared to those in the control group. However, these parameters had no significant effect on gestational age and birth weight. In the study by Kinomoto-Kondo and cols. (11), a negative correlation was found between the first trimester maternal FT4 levels and gestational age.

In the studies evaluating the effects of thyroid functions on pregnancy, it has been reported that an increase in maternal FT4 level in pregnant women achieving euthyroid state or having GTT negatively affects gestational age and birth weight (11-13). However, in the present study, there was no correlation between the FT4 level and gestational age and birth weight. In the FaSTER study, the negative correlation between maternal FT4 levels and birth weight in pregnant women achieving euthyroid state was also confirmed (14). However, the effects of FT3 level and FT3/FT4 ratio on this analysis were not investigated; this was stated as the limitation of the study. It was also emphasized that maternal FT3/FT4 ratio was a stronger metabolic indicator than FT4 alone (14). In the present study, when the effect of FT3/FT4 ratio on birth weight and gestational age was evaluated, a positive correlation was found between the maternal FT3/FT4 ratio and birth weight in the GTT group. In the FaSTER study, FT4 level was negatively correlated with the maternal age and weight (14). Considering this situation, the correlations of FT4 and FT3/FT4

ratio with the maternal age and weight were also examined in the present study groups. A positive correlation was found between the FT3/FT4 ratio and the maternal weight in the group with GTT. To the best of our knowledge, there has been no analysis on this issue in patients with GTT in the literature. When the correlation analysis was performed considering the effect of maternal weight as a confounding factor, a positive correlation was obtained between the FT3/FT4 ratio and the birth weight in our GTT group. This result could help us to explain the normal birth weight in our patients with GTT. As one of the plasma thyroid hormone levels increases, the other decreases. FT3 is an active thyroid hormone and stimulates endogenous glucose production. This is related with relatively decreased FT4 level and increased maternal glucose production (15).

The major limitation of the present study is being a single center and small-scale study. On the other hand, in accordance with the literature, regular follow-up of the patients owing to our pregnancy tracking system is one of the advantageous of the present study.

In conclusion, no significant difference was found between the birth weight and gestational age when comparing the patients with GTT with the healthy controls. On the other hand, maternal FT3/FT4 ratio had a positive correlation with the birth weight in the GTT group. Maternal characteristics (age, weight, BMI, etc.) and FT3/FT4 ratio should also be taken into account when conducting an impact assessment study in patients with GTT. Future multi-center prospective studies are needed on this subject.

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Effects of concomitant obesity and diabetes on the aggressiveness and outcomes of differentiated thyroid cancer patients

Onur Elbasan[1]
https://orcid.org/0000-0001-8580-9471

Dilek Gogas Yavuz[1]
https://orcid.org/0000-0002-0075-6313

[1] Division of Endocrinology and Metabolism, Marmara University College of Medicine, Istanbul, Turkey

ABSTRACT

Objective: Obesity and diabetes are the risk factors for cancer development including differentiated thyroid cancer (DTC). Contradictory accumulated data indicates the possible negative effects of obesity and hyperglyceamia as a factor for aggressiveness of DTC.The aim of the present study is to investigate the association of high body mass index (BMI) and presence of type 2 diabetes mellitus (T2DM) on the histological aggressiveness and clinical outcomes in DTC patients followed for over 4 years in a single center. **Materials and methods:** Consequative 526 DTC patients who had undergone total thyroidectomy and/or radioactive iodine (RAI) ablation were reviewed retrospectively. Patients were divided into groups based on their BMI: normal weight, overweight, obese and also were evalauted in 3 groups presence of diabetes, prediabetes and nomoglyceamia. Histological aggressiveness of DTC at the time of diagnosis and clinical response at the time of last clinical visit were reassessed according to the criteria suggested by ATA 2015 guideline. **Results:** No differences in histopathologic features, risk of recurrence, cumulative dose of RAI ablation and prevalence of 131I avid metastatic disease were demonstrated among the groups both classified according to BMI and hyperglycemia. Mean of 3.4 year follow-up also showed no differences in the clinial repsonse to therapy and percentage of nonthyroid primary cancer in DTC patients. **Conclusion:** In this retrospective study we demonstrated that obesity and T2DM have no additive effect on DTC aggressiveness and response to therapy. DTC patients with obesity and diabetes can be treated according to present guidelines without requirement for spesific attention. Arch Endocrinol Metab. 2021;65(4):455-61

Keywords
Differantiated thyroid cancer; BMI; obesity; prediabetes; T2DM; metastasis

Correspondence to:
Onur Elbasan
Division of Endocrinology and Metabolism, Marmara University Pendik Training and Research Hospital Fevzi Cakmak, Muhsin Yazicioglu Street No: 10, 34899 Pendik, Istanbul, Turkey
dronurelbasan@hotmail.com

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INTRODUCTION

Thyroid cancer is one of the most common endocrine malignancies and its incidence rate has increased significantly in the last decades. According to 2016 standardized data in Turkey, the frequency of thyroid carcinoma was 62-229 per 1,000,000 persons (1). Although increased incidence of thyroid carcinoma is attributed to rising awareness and easier diagnosis, accumulating data have suggested that concomitant diseases such as diabetes and obesity may play a role (2-5). Obesity and type 2 diabetes mellitus (T2DM) are complex metabolic disorders, and epidemiological studies have indicated their association with increased risks for several cancers including colon, breast, pancreas, liver, endometrial and thyroid (6,7). The possible mechanisms with increased thyroid cancer risk in obesity and diabetes are still unclear. Hyperinsulinemia, insulin resistance and proinflamatory state may have an effect on thyroid carcinogenesis through insulin receptors overexpressed in cancer cells (4,5,8). According to different studies, obese and diabetic patients may have an increased risk of malignancy and differentiated thyroid cancer (DTC) aggressiveness as a result of clinically higher serum thyroid stimulating hormone (TSH) levels compared to the normal population (9-11).

Body mass index (BMI) has been linked to a higher incidence of thyroid cancer in some cohorts (2,3). Some studies have found that obesity is associated with more advanced stage or aggressive cancers at presentation and the recurrence or metastasis for several types of cancers, but it has not been confirmed

with recent studies for thyroid cancer (12-15). Along with the studies indicating an increased risk of thyroid cancer in diabetic patients (4,5), there are also studies showing the lack of association (16-18). Retrospective and prospective clinical studies reported conflicting results and the effects of obesity and diabetes on the clinical outcomes of thyroid cancer have not yet been clarified. In the present study, we aimed to investigated the associations of BMI, prediabetes and T2DM with pathological features and clinical outcomes of DTC patients followed for over 4 years in a single center.

MATERIALS AND METHODS

The Study Protocol was approved by the ethics committee of Marmara University School of Medicine (protocol number: 09.2018.494). The study was conducted in accordance with the Declaration of Helsinki. In this retrospective study consequtive 526 DTC patients followed-up from January 2010 to December 2018 were included in the study. Patients who had undergone total thyroidectomy and diagnosed with papillary, follicular and hurtle cell thyroid carcioma through pathologic examination of the surgical specimen were enrolled. Demographic parameters, histopathological findings, surgical reports, radioactive iodine ablation history, radiologic evaluation reports after surgery (neck ultrasonography, iodine scans), clinical data [duration of disease, follow-up time, daily dose of levothyroxine, history of obesity, prediabetes and T2DM, weight, height, body mass index (BMI:calculated by weight/height^2)] were recorded. Avaible biochemical data of fasting plasma glucose (FPG), HbA1c levels at the time of diagnosis, and thyroid stimulating hormone (TSH), free thyroxine (T4), thyroglobulin (Tg) and thyroglobulin antibody (TgAb) values after surgery and also at the last visit were recorded. Patients were categorized in three groups according to their BMI: normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (BMI ≥ 30 kg/m^2), and were also classified in three groups according to their glycemic status considering diabetes history, FPG, Hba1c values as normoglycemia (FPG < 100 mg/dL and/or HbA1c < 5.7), prediabetes (FPG = 100-125 mg/dL and/or HbA1c = 5.7%-6.4%) and diabetes (FPG ≥ 126 and/or HbA1c ≥ 6.5) according to the ADA 2019 guideline. The clinicopathological findings were compared in the groups were categorized according to BMI and glycemic disorders. As a routine procedure, patients were periodically followed-up at regular intervals with biochemical and ultrasonographic evaluations, and the follow-up interval and other diagnosis and/or treatment options were determined according to the dynamic risk reclassification process (19). Thyroglobulin values were taken into account only in the absence of thyroglobulin antibodies (TgAb) to elicit clinical response. Changes in levels over time were accepted to describe the response to treatment in the presence of TgAb (20). Development of new metastatis after surgical removal, RAI ablation requirement, cumulative RAI doses, nonthyroid primary cancer were recorded for the whole follow up period.

Risk evaluation at the time of diagnosis

Stage and risk of metastasis/recurrence were evaluated according to the American Joint Committee on Cancer (AJCC) 7th Edition Staging System for Differentiated Thyroid Carcinoma using the AMES (Age, Metastases, Extent, Sex) score at the time of diagnosis (21). We also stratified the risk of recurrence based on the ATA 2009 guideline as Low, Intermediate and High categories (19).

Radioactive iodine ablation treatment

Thyroid remnant ablation was performed 4-6 weeks after surgery. The 131I ablation dose for almost all patients was 100 mCi (millicuries). A whole body scan (WBS) was performed 1 week after 131I administration. The first 131I treatment was conducted after recombinant human TSH stimulation (rhTSH) in 70.3% of patients (n:370) and after 3-4 weeks of withdrawal of L-thyroxinere placement therapy in 29.7% of patients (n:156). TSH stimulation was obtained by administering 1 injection of rhTSH on 2 consecutive days. 131I neck take-up was measured for diagnostic purposes at 3 and 24 hours after the administration of 0.05 mCi of 131I. Based on the radioiodine enhancement, we distinguished the post-therapeutic WBS results as negative; when the presence of 131I uptake was exclusively present in the thyroid remnant or positive; for the presence of uptake related to lymph nodes and/or distant metastases.WBS requirement in follow-up was determined according to risk reassessment.

Reassesment of clinical response

The clinical response was assessed at the time of the last outpatient clinic visit, according to the criteria

suggested by the 2015 ATA guideline with evaluation of postoperative Tg and imaging methods (22). The patients were evaluated according to the final response to therapy as excellent, biochemical incomplete, structural incomplete and indeterminate.

Biochemical Parameters

Serum TSH (N: 0.34-5.6 μIU/mL), free T4 (N: 0.61-1.12 ng/dL), Tg and TgAb was measured in automated serum samples by paramagnetic particle chemiluminescence immunoassay method (DxI800, Beckman Coulter, USA). Serum glucose levels were automatically measured by the hexokinase method (AU5800, Beckman Coulter, USA). HbA1c percentages were measured in EDTA whole blood samples by boranetafinite chromatography (TrinityBiotech, Ireland). Tg ≤ 0.2 andTgAb ≤ 0.9 values were considered as remission.

Statistical analysis

The distribution of the data was analysed by Shapiro-Wilk Test. One-Way Anova test was used for comparing the data showing normal distribution among three groups. The comparing of the variables that do not have normal distribution among three groups was analysed by Kruskal-Wallis ANOVA Test. Fisher Exact Test were applied for analysing the differences between categoric data. Post-hoc comparings of the variables that were found meaningful after Anova and Kruskal Wallis tests were analysed by Dunn test. We gave frequency for the following descriptive statistics of data for numerical variables: average, standard deviation, median, minimum, and categorical variables. All analyses were made by IBM Statistics 22.0 Program within the 0.05 significance level.

RESULTS

The epidemiological and clinicopathological features of study population are shown in Table 1. Of 526 DTC cases involved in the study, 512 (97.34%) were papillary thyroid carcinoma, 12 (2.28%) were follicular and 2 (0.38%) were Hurthle cell carcinoma. The median age at evaluation was 51 years and the patients were predominantly female (81%). The mean BMI was 30.55 ± 4.88 kg/m^2; 53% of the cases were obese, 37.3% were overweight and 9.7% were normal weight. T2DM frequency was 19.6%, 31.7% of the patients

Table 1. Clinical, histopathologic and loboratory characteristics of DTC patients

Parameter	N (%)
Age (years)	51 (19-88)
Gender (female/male)	426 (81%)/100 (19%)
Age at diagnosis (years)	44.55 ± 12.65
Duration of disease (years)	4 (1-37)
Follow-up time (years)	3.4 ± 2.58
Daily dose of LT4 (mcg)	119.63 (25-300)
LT4 dose per kilogram (mcg/kg)	1.62 ± 0.52
Cumulative RAI dose (mCi)	100 (30-600)
Nonthyroid primary cancer	44 (8.4%)
Histology	
Papillary carcinoma	512 (97.34%)
Follicular carcinoma	12 (2.28%)
Hurtle cell carcinoma	2 (0.38%)
Body mass ındex (kg/m^2)	30.55 ± 4.88
Obese/overweight/normal weight	279 (53%)/196 (37.3%)/51 (9.7%)
Glycemic Status	
T2DM/prediyabetes/normoglycemia	103 (19.6%)/167 (31.7%)/ 256 (48.7%)
Pathology	
Tumor size (mm)	11 (4-110)
Multicentric tumor (n)	159 (30.23%)
Lymphatic invasion (n)	67 (12.7%)
Vascular invasion (n)	75 (14.2%)
Extrathyroidal soft tissue invasion	99 (18.8%)
Metastasis	95 (18.1%)
Cervical lymph node metastasis	81 (15.4%)
Distant metastasis	14 (2.7%)
Laboratory	
TSH (μIU/mL)	0.4 (0.001-121)
Free T4 (ng/dL)	1.07 (0.06-5.15)
Thyroglobuline (ng/mL)	0.2 (0.04-4154)
AMES risk (AJCC 7th edition)	
High	274 (52.1%)
Low	252 (47.9%)
Risk of recurrence (ATA)	
High	24 (4.6%)
Intermediate	166 (31.5%)
Low	336 (63.9%)
Staging	
I	343 (65.2%)
II	13 (2.5%)
III	137 (26%)
IV	33 (6.3%)
Response to therapy	
Excellent	239 (45.5%)
Biochemical incomplete	36 (6.8%)
Sutructural incomplete	17 (3.2%)
Indetermine	234 (44.5%)

were prediabetic and 48.7% were normoglycemic. The rate of metastasis was 18.1% and 52.1% of the patients were at high risk according to the AMES score. The risk of recurrence was found to be low in more patients (63.9%) according to ATA, and 65.2% of the patients were classified as Stage 1 based on the TNM system.

Clinical and demographic features according to BMI are presented in Table 2. Obese and overweight groups have a higher age than the overweight and normal weight groups ($p = 0.003$). The age at diagnosis in the obese group was also significantly higher than the other groups ($p = 0.017$). There was no difference between the groups in terms of histopathological features such as tumor size, multicentricity, lymphatic, vascular and extrathyroidal invasion and laboratory parameters such as TSH, free T4 and Tg. There was no significant difference between the groups in terms of metastasis, AMES and ATA high risk patient ratio. The rate of Stage 1 cases and patients with excellent response to therapy was also similar in groups. Cumulative RAI dose and nonthyroid primary cancer rates did not differ between BMI groups.

Clinicopathological features among three groups according to glycemic status are demonstrated in Table 3. The mean age and age at DTC diagnosis of the diabetic patients were significantly higher than other groups ($p < 0.001$, $p < 0.001$). The female gender ratio and BMI were found to be significantly higher in the prediabetic group compared to the other groups ($p = 0.043$, $p < 0.001$, respectively). Tumor diameter did not differ between groups. There was also no significant difference between the groups in terms of multicentricity, tumor invasion parameters and metastasis characteristics. Also, TSH, free T4 and Tg levels were not significantly different between groups. There was no significant difference between the groups regarding the rate of patients with AMES and ATA high risk, Stage 1, and excellent response to treatment. Cumulative RAI doses and the rate of nonthyroid primary cancer were similar in all groups.

Table 2. Clinical, laboratory and histopathologic evalauation of DTC patients according to BMI

	Normal weight (n = 51)	Overweight (n = 196)	Obese (n = 279)	p
Age (years)	46.18 ± 13.7	49.76 ± 13.11	52.18 ± 11.98	**0.003**
Female (n)	38 (74.51%)	141 (71.94%)	247 (88.53%)	**<0.001**
Age at DTC diagnosis (years)	40.86 ± 13.76	43.67 ± 13.18	45.84 ± 11.9	**0.017**
Duration of disease (years)	4 (1-17)	3 (1-37)	4 (1-30)	0.415
Daily dose of LT4 (mcg)	103.57 (57.14-200)	116.06 (50-235.71)	125 (25-300)	**0.023**
Tumor size (mm)	11 (1-85)	11.5 (1-80)	11 (0.4-110)	0.821
Multicentric tumor (n)	13 (25.49%)	68 (34.69%)	78 (27.96%)	0.215
Lymphatic invasion (n)	10 (19.61%)	28 (14.28%)	32 (11.47%)	0.297
Vascular invasion (n)	9 (17.65%)	28 (14.28%)	38 (13.62%)	0.796
Extrathyroidal soft tissue invasion (n)	10 (19.61%)	38 (19.39%)	51 (18.28%)	0.894
All metastatis	12 (23.53%)	41 (20.92%)	42 (15.05%)	0.148
Cervical lymph node metastasis	11 (21.57%)	34 (17.35%)	37 (13.26%)	0.525
TSH (μIU/mL)	0.83 (0.01-45.98)	0.28 (0.001-121)	0.47 (0.01-100)	0.069
Free T4 (ng/dL)	1.04 (0.41-1.76)	1.1 (0.06-3.06)	1.05 (0.16-5.15)	0.321
Tg (ng/mL)	0.2 (0.2-25.2)	0.2 (0.04-2475)	0.2 (0.04-4154)	0.053
AMES high risk	34 (66.67%)	102 (52.04%)	138 (49.46%)	0.104
High risk of recurrence (ATA)	1 (1.96%)	11 (5.61%)	12 (4.3%)	0.791
Stage 1	33 (64.7%)	133 (67.86%)	177 (63.44%)	0.350
Excellent response to therapy	20 (39.42%)	92 (46.94%)	127 (45.52%)	0.880
Cumulative RAI dose (mCi)	100 (50-400)	100 (30-600)	100 (30-400)	0.916
Nonthyroid primary cancer	4 (7.84%)	17 (8.67%)	23 (8.24%)	0.976

DTC: differentiated thyroid cancer; LT4: L-thyroxine; TSH: thyroid stimulating hormone; T4: thyroxine; Tg: thyroglobuline; ATA: American Thyroid Association; AJCC: American Joint Committee on Cancer; AMES: age, metastases, extent, sex; RAI: radioactive iodine ablation.

Table 3. Clinical, laboratory and histopathologic evaluation of DTC patients according to glycemic status

	Normoglycemia (n = 256)	Prediabetes (n = 167)	Diabetes (n = 103)	p
Age (years)	46.25 ± 13.02	52.6 ± 10.56	59.3 ± 9.89	**<0.001**
Female(n)	209 (81.64%)	142 (85.03%)	75 (72.82%)	**0.043**
BMI (kg/m^2)	29.3 (17.3-53.67)	31.63 (18.73-58.25)	31.24 (17.72-47.42)	**<0.001**
HbA1c (%)	5.3 (4.2-5.6)	5.9 (4.4-6.4)	6.5 (5.0-13.4)	**<0.001**
Age at DTC diagnosis (years)	39 (13-76)	46 (23-78)	53 (8-78)	**<0.001**
Duration of DTC (years)	3 (1-29)	5 (1-30)	4 (1-37)	**0.006**
Daily dose of LT4 (mcg)	121.42 (25-300)	114.28 (50-271.42)	128.57 (25-300)	0.082
Tumor size (mm)	12 (1-110)	12 (1-80)	10 (0.4-70)	0.05
Multicentric tumor (n)	88 (34.38%)	44 (28.35%)	27 (26.21%)	0.131
Lymphatic invasion (n)	36 (14.06%)	19 (11.38%)	12 (11.65%)	0.418
Vascular invasion (n)	41 (16.02%)	23 (13.77%)	7 (6.8%)	0.066
Extrathyroidal softtissue invasion (n)	50 (19.53%)	32 (19.16%)	17 (16.5%)	0.502
All metastatis	51 (19.92%)	24 (14.37%)	20 (19.42%)	0.322
Cervical lymph node metastasis	43 (16.8%)	22 (13.17%)	17 (16.5%)	0.930
TSH (µlU/mL)	0.35 (0.001-56.04)	0.4 (0.01-121)	0.44 (0.02-59)	0.267
Free T4 (ng/dL)	1.08 (0.25-3.59)	1.06 (0.06-5.15)	1.07 (0.16-4.58)	0.698
Tg (ng/mL)	0.2 (0.04-2475)	0.2 (0.04-558)	0.2 (0.04-4154)	0.259
AMES high risk	140 (54.69%)	89 (53.29%)	45 (43.69%)	0.157
High risk of recurrence (ATA)	12 (4.69%)	8 (4.79%)	4 (3.88%)	0.952
Stage 1	181 (70.7%)	104 (62.28%)	58 (56.31%)	0.087
Excellent response to therapy	118 (46.09%)	73 (43.71%)	48 (46.6%)	0.150
Cumulative RAI dose (mCi)	100 (30-600)	100 (50-300)	100 (30-600)	0.823
Nonthyroid primary cancer	17 (6.64%)	18 (10.78%)	9 (8.74%)	0.320

BMI: body mass index; DTC: differentiated thyroid cancer; LT4: L-thyroxine; TSH: thyroid stimulating hormone; T4: thyroxine; Tg: thyroglobuline; ATA: American Thyroid Association; AJCC: American Joint Committee on Cancer; AMES: age, metastases, extent, sex; RAI:r adioactive iodine ablation.

DISCUSSION

In this retrospective study, we observed no additive effect of the presence of obesity and T2DM on the histological and clinical aggressiveness of DTC patients followed in a single center. This result is concordant with numerous studies in the literature, although a few clinical studies have reported contrary results. In a study conducted in a large group of patients, there was no significant relationship between BMI and tumor size, multifocality, extrathyroidal invasion, cervical lymph node metastasis or distant metastasis (13). In a retrospective study, Paes and cols. reported a lack of association between BMI and histological aggressiveness of DTC features (12). Similarly, in a Polish study including 1181 patients, BMI was not found to be a risk factor for the aggressiveness of DTC (15). A Korean series further showed that obesity influenced larger tumor size, the presence of extrathyroidal invasion and advanced TNM stage (23). We did not show any histopathological and clinical difference between obese, overweight and normal weight patients in terms of tumor size, multicentricity, tumor invasion characteristics and metastasis ratio in accordance with the literature, as many clinical studies did not demonstrate tumor size and invasiveness according to BMI in DTC patients (12,13,24). In another research, patients were evaluated according to the ATA risk stratification system and significant relationship was not found between BMI and ATA risk score (14). In an Italian cohort, obese patients had less Stage I disease, compared to overweight and normal weight patients, but it was not statistically significant. In the same study, there was also no difference between the groups in terms of the response to therapy (24). Also in our study, the rate of Stage 1 disease was lower in the obese group, which was not statistically significant.

Patients with AMES and ATA high risk score, and who had excellent response to treatment were also similar between obese, overweight and normal weight groups. In our study, the rate of overweight (37.3%) and obese (53%) patients is higher compared to the other studies mentioned above that examined the effect of obesity on thyroid cancer.

DTC aggressiveness was less invastigated in diabetic and prediabetic patients. A prospective study indicated that the worse pathological features were higher in diabetic patients than nondiabetics (25). However, in a study including 8-year follow-up (26), no significant difference was observed between the diabetic and control groups in terms of clinicopathological characteristics. In a retrospective study, it was mentioned that T2DM was associated with the advanced TNM stage in DTC cases (27), whereas another study comparing diabetic and nondiabetic patients by matching them exactly did not find a difference regarding clinical stages (25). Although the rate of stage 1 disease was lower in the prediabetic and diabetic groups compared to the normoglycemic group in our study, we did not observe a significant difference. The risk of recurrence based on ATA and AMES scores was also similar in all groups (Table 3). We could not find any difference of DTC cancer aggressiveness in diabetic and prediabetic patients compared to normoglycemic ones. We can state that the low median HbA1c values (6.5%) of the diabetic patients in our study, that is the higher rate of controlled diabetes compared to other studies mention above, may contribute to this result.

Metastasis in DTC was also previously evaluated in diabetic and nondiabetic patients, and T2DM has been shown to rise the metastasis (25,27). In contrast, no significant difference was found between the normoglycemic, prediabetic and diabetic groups in terms of metastasis and response to therapy in our study. This may be related to the generally low metastasis rate in this study compared to others. It can be considered the fact that the initial dose of RAI ablation was 100 mCi in almost all of our patients is effective on low metastasis rates, clinical outcome and response to therapy.

Since the increasing effects of obesity and T2DM on other types of cancer are known (27), we also examined other nonthyroidal cancers in our study and found that the rate of nonthyroid primary cancer was similar in all groups according to BMI and glycemic status.

In our research, the rate of obesity was 53% and the rate of T2DM was 19.6% and these rates are higher compared to our country and other studies (12-14,23-29). In spite of these high rates of diabetes and obesity, we have reported that they have no effect on histopathological characteristics and the outcome of thyroid cancer. The limitations of this study can be stated as followes: The retrospective structure, patient heterogeneity, not exactly knowing the duration of T2DM and prediabetes, the difficulty of separating and analyzing obesity and T2DM which are intertwined conditions, and the lack of clear knowledge of the effects of the drugs on weight and glycemic status.

In conclusion, according to our study results of Caucasian subjects followed-up in a single center, we demonstrated that the presence of obesity and diabetes have no additive effect on the aggressiveness and response to therapy in DTC patients. DTC patients with obesity and diabetes may be treated according to present guidelines with no specific attention requirement.

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Are overweight and obesity risk factors for invasive mechanical ventilation in severe coronavirus disease 2019 pneumonia?

[1] Department of Internal Medicine, American British Cowdray Medical Center, Mexico City, Mexico
[2] Research Unit in Endocrine Diseases, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico City, Mexico

Maria Fernanda Coss-Rovirosa[1]
https://orcid.org/0000-0003-3739-3453

Mercedes Aguilar-Soto[1]
https://orcid.org/0000-0002-3522-8406

Dalia Cuenca[1]
https://orcid.org/0000-0001-9961-4667

Mariana Velez-Pintado[1]
https://orcid.org/0000-0002-7834-4917

Antonio Camiro-Zuñiga[1]
https://orcid.org/0000-0003-0957-9509

Aldo Ferreira-Hermosillo[2]
https://orcid.org/0000-0002-5159-9856

Moises Mercado[2]
https://orcid.org/0000-0002-4748-9734

Correspondence to:
Moisés Mercado
Av. Cuauhtémoc 330,
Doctores, Cuauhtémoc
06720 – Mexico City, Mexico
moises.mercado@endocrinologia.org.mx

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ABSTRACT

Objective: Describe the demographic, clinical, and biochemical characteristics of overweight or obese people with severe COVID-19 pneumonia and evaluate its association with mechanical ventilation requirements in a Mexican cohort. **Subjects and methods:** Data were obtained from medical electronic records. Patients were divided in three groups according to the World Health Organization (WHO) classification of body mass index (BMI): lean, overweight and obese. Baseline characteristics and clinical course were compared among these 3 groups. **Results:** The study included a total of 355 patients with confirmed COVID-19 diagnoses. Patients with obesity and overweigh, according to the WHO classification, had no significantly increased risk of requiring intubation and invasive mechanical ventilation (IMV) compared to lean subjects, with an odds ratio (OR) of 1.82 (95% CI, 0.94-3.53). A post hoc and multivariate analysis using a BMI > 35 kg/m^2 to define obesity revealed that subjects above this cut off had as significantly increased risk of requiring IMV after with an OR of 2.86 (95% CI, 1.09-7.05). **Conclusions:** We found no higher risk of requiring IMV in patients with overweight or obesity while using conventional BMI cutoffs. According to our sensitivity analyses, the risk of IMV increases in patients with a BMI over 35 kg/m^2. Arch Endocrinol Metab. 2021;65(4):462-7

Keywords
Overweight; obesity; COVID-19; invasive mechanical ventilation (IMV)

INTRODUCTION

In December 2019, the new coronavirus SARS-CoV-2 was described for the first time in China's Wuhan province (1). In under 4 months, the virus caused the greatest pandemic of the century.

By September 27, 2020, the World Health Organization (WHO) had reported nearly 33 000 000 cases and over 900 000 deaths worldwide most of them on the American continent. Mexico has reported 817 000 cases since its first case in February 2020 and a mortality rate of 11%, higher than the worldwide average (2).

An age over 65 years and the presence of comorbidities are the main risk factors for hospitalization (3). Diabetes, hypertension, and obesity comprise the most common preexisting conditions (4). Obesity, defined as a body mass index (BMI) of > 30 kg/m^2, commonly appears in COVID-19 patients, with a 47% prevalence in patients requiring hospitalization (5). Previous studies demonstrate that patients with obesity have a high risk for a severe disease. These patients often require invasive mechanical ventilation (IMV) and have a higher mortality rate than non-obese patients.

In fact, a one-unit increase in BMI likely increases the risk of severe disease by 12% (6). One cohort study showed that obesity (BMI ≥ 30 kg/m²) is a strong, independent, and clinically relevant risk factor for severe illness leading to intubation or death (7).

In Mexico, obesity's prevalence has risen substantially in the last 30 years and now affects over 35% of the adult population (8). Obesity associates with other conditions, including cardiovascular disease, type 2 diabetes mellitus, cancer, obstructive sleep apnea, and more (9). Aside from these metabolic complications, patients with obesity have a chronic inflammatory state (10). mediated by the adipose tissue's secretion of proinflammatory cytokines, such as adiponectin, interleukin-6 (IL-6), interleukin-1, and tumor necrosis factor-α.

Thus, patients with obesity have an altered innate immune response that negatively affects COVID-19 outcomes (11). We describe the demographic, clinical, and biochemical characteristics of patients with obesity and overweight and evaluate the association with an IMV requirement.

SUBJECTS AND METHODS

Study design and setting

From March 12 to July 15 of 2020, we collected information from patients admitted to the American British Cowdray Medical Center, a private teaching hospital in Mexico City. Clinical, biochemical, and imaging information was obtained and analyzed retrospectively. We included patients 18 years old or older who had a documented diagnosis of COVID-19 (defined as a positive PCR for SARS-CoV2 or a chest CT scan showing characteristics of COVID-19 pneumonia). Data came from the hospital's electronic medical records. We included all patients with height, weight, and outcome data; we excluded patients with missing values. The scientific and ethics committees of the medical center approved the protocol, and the study followed the principles of the Helsinki declaration.

Study procedures

As stated before, a COVID-19 diagnosis was defined as a positive PCR for SARS-CoV2 or a chest CT scan showing characteristics of COVID-19. We obtained samples for SARS-CoV-2 testing according to the Centers for Disease Control guidelines. Nucleic acid was extracted with the RNeasy Mini Kit (Qiagen), and we used a 7500 Real-Time PCR System (Applied Biosystems) targeting the *CoV E* gene and the *CoV RdRP* gene for nucleic acid amplification. Positive chest CT scans occurred when characteristics of COVID-19 were verified by a consensus among several radiologists.

Patients were classified according to the WHO classification of overweight and obesity. A normal BMI is between 18.5 and 24.9 kg/m², an overweight BMI ranges from 25-29.9 kg/m², and obesity BMI is > 30 kg/m². The main outcome was mechanical ventilation requirement at admission or during hospitalization, as recorded in electronic medical records. We also evaluated days of hospital stay as a secondary outcome.

Statistical analysis

Depending on data distribution (via the Shapiro Wilks test), continuous variables are described as means with standard deviations (normally distributed) or as a median interquartile range (abnormally distributed). We used frequencies and simple proportions to summarize categorical variables. We used ANOVA to compare groups with normal BMI patients, patients with overweight, and patients with obesity. We conducted univariate, age- and sex-adjusted, and multivariate logistic regressions to evaluate the need for IMV in normal, overweight, and patients with obesity. We also adjusted for possible confounders, such as C-reactive protein levels, oxygen-saturation levels, and mean arterial pressure on admission. We conducted a linear regression analysis using continuous BMI to assess the days of a hospital stay. The same covariates were used for the logistic regression analysis.

Few patients had a normal BMI, so we conducted a sensitivity analysis defining the reference group as patients with a normal or overweight BMI. We also conducted a sensitivity analysis, defining obesity as a BMI over 35 kg/m². All statistical tests were 2-sided, and a p-value < 0.05 was considered statistically significant. We used SAS University Edition version 9.4 statistical software (SAS Institute, Cary, NC).

RESULTS

This study included a total of 355 patients with confirmed COVID-19 diagnoses. Table 1 depicts the baseline characteristics of patients, who were divided in three groups according to their BMI. Of the patients, 23% (n = 82) had normal BMI, 45% (n = 160) were

Table 1. Baseline characteristics of patients divided by BMI classification

	Categories of weight			p value
	Normal (n=82)	Overweight (n=160)	Obesity (n=113)	
BMI	23.4 (22.2-24.4)	27.4 (26.1-28.4)	33.7 (31.6-36.4)	<0.0001
Age, years	56.8 (17.3)	53.3 (15.7)	50.8 (13.2)	<0.0001
Women n, (%)	33 (40)	54 (34)	33 (29)	0.28
Prediabetes n, (%)	1 (2)	9 (7)	5 (5)	0.27
Diabetes n, (%)	15 (19)	25 (16)	21 (19)	0.83
Hypertension n, (%)	21 (26)	42 (27)	36 (32)	0.53
Severity Scales				
NEWS (11)	5 (4-7)	6 (4-7)	6 (5-8)	0.11
MULBSTA (12)	7 (5-10)	9 (5-11)	7 (5-11)	0.67
CALL-SCORE (13)	7 (5-10)	7 (6-10)	7 (6-10)	0.79
Laboratory values				
Leucocytes (10^3/uL)	6 (5-9)	7 (5-10)	6 (5-8)	0.03
Neutrophils (10^3/uL)	5 (3.1-7.5)	5.9 (3.7-8.3)	5 (3-7)	0.18
Lymphocytes (10^3/uL)	1.0 (0.76-1.37)	0.99 (0.66-1.46)	1.02 (0.80-1.4)	0.75
Neutrophil/Lymphocyte Ratio	4.8 (2.7-8)	5.6 (3-9.4)	4.8 (2.8-7.7)	0.35
Glucose (mg/dL)	115 (99-131.8)	116 (98.4-134.9)	114 (97-132)	0.77
HDL-c (mg/dL)	33.5 (19-40)	35 (28-42)	31 (27-39)	0.256
DL-c (mg/dL)	58.5 (31-79)	66.5 (47.5-83)	69 (52-93)	0.03
Triglycerides (mg/dL)	156 (80-156)	126 (95-176)	130 (100-183)	0.018
CRP (mg/dL)	10.5 (4-17.5)	12 (6-22)	11 (4-20)	0.27
Ferritin (ng/mL)	762 (337-1385)	897 (435-1623)	1075 (665-1602)	0.11
IL-6 (pg/mL)	25.5 (16.5-54.5)	38 (16-67)	39 (15-63)	0.57
D-dimer (ng/mL)	1000 (760-2320)	790 (529-1219)	796 (505-1256)	0.11
Treatment				
Lopinavir/Ritonavir n, (%)	39 (49)	109 (68)	68 (61)	0.01
Azithromycin n, (%)	58 (72)	117 (74)	92 (82)	0.17
Hidroxicloroquine n, (%)	63 (72)	126 (80)	92 (81)	0.31
Glucocorticoids n, (%)	6 (7)	13 (8)	5 (4)	0.35
Tocilizumab n, (%)	21 (27)	66 (44)	48 (46)	0.02
Outcomes				
Required ICU n, (%)	9 (13)	24 (16)	24 (22)	0.15
Admitted to ICU n, (%)	31 (38)	56 (35)	37 (33)	0.26
Required mechanical ventilation n, (%)	22 (27)	56 (35)	43 (38)	0.25
Death (%)	7 (9)	13 (8)	4 (4)	0.25

1 Values are percentages, mean ± SD or median (IQR) as appropriate HDL-c = High density lipoprotein, LDL = Low density lipoprotein, CRP = C-reactive protein, IL-6 = Interleukin 6.

overweight, and 32% (n = 113) were patients with obesity. The median BMI for each group was 23.4 (22.2-24.4) kg/m², 27.4 (26.1-28.4) kg/m², and 33.7 (31.6-36.4) kg/m², respectively. The mean age was significantly lower (p < 0.0001) in patients with obesity (50.8 ± 13.2 years) compared to those with a normal BMI (56.8 ± 17.3 years). The normal BMI group had a higher proportion of women (40%), with lower percentages of women for overweight (34%) or obesity (29%).

The prevalence of prediabetes (2% of normal BMI patients, 7% of overweight patients, and 5% of patients with obesity, p=0.27), diabetes (normal BMI = 19%, overweight = 16%, obese = 19%, p = 0.83) and

hypertension (normal BMI = 26%, overweight = 27%, obese = 32%, p = 0.53) was similar among all groups. Other important comorbidities had a prevalence of 2% for chronic kidney disease, 2.4% for heart disease, and 2% for chronic obstructive pulmonary disease without significant differences among groups (p = 0.38, 0.6, and 0.37, respectively). Cancer's prevalence differed significantly, with 9% in lean patients, 1% in patients with overweight and 4% in patients with obesity (p = 0.015). No between-group difference in severity existed according to validated scores, such as the National Early Warning Score (12) (p = 0.11), MuLBSTA (13) (p = 0.67), and CALL (14) (p = 0.79) scores. Laboratory data were obtained on admission for all values for all patients. The normal BMI and patients with obesity groups had lower leucocyte counts compared to the overweight group (p = 0.03). The groups had similar levels of other inflammatory markers (lymphocyte count, D Dimer, C reactive protein, ferritin, and interleukin-6).

The 3 groups had similar HDL-cholesterol levels, but patients with obesity had higher LDL-c levels. Patients with normal BMI had higher triglyceride levels compared to those who were overweight or obese. All patients were treated with supplemental oxygen by regular nasal cannula or mask upon admission to the hospital. Oxygen was administered via high-flow nasal cannula to 17% of lean individuals, 48% of patients with overweight and 36% of patients with obesity (p = 0.30). Continuous positive airway pressure was used for 5 patients with overweight and 5 patients with obesity. The revised version of the manuscript specifies those details. Secondary infections did not differ between the 3 groups. Bacterial pneumonia was diagnosed in 8% of the patients, without a difference among groups (p = 1). Invasive pulmonary aspergillosis had an overall incidence of 4.2% and was similar between groups (p = 0.33). No significant differences exist in ICU admission, the need for mechanical ventilation, or mortality rate (6% in the general group). The median number of days from symptom onset to hospitalization was 8.5 (6-12, p = 0.33).

Compared to patients with normal BMI, patients with overweight and obesity were more likely to require IMV, but the risk only remained significant for patients with obesity when adjusting for age and sex (OR 1.97, 95% CI, 1.02-3.79). The risk was no longer significant in the multivariate analysis (OR 1.82, 95% CI, 0.94-3.53; Table 2).

When conducting sensitivity analyses with normal BMI and patients with overweight together as reference, patients with obesity had a higher risk of requiring IMV and an increased OR after multivariable adjustment (OR 2.04, 95% CI, 1.09-3.81). Considering obesity as having a BMI > 35 kg/m^2, analyses showed an increased risk of requiring IMV after a multivariate adjustment with an OR of 2.86 (95% CI, 1.09-7.05; Table 3).

Increased BMI associated with a longer hospital stay according to a bivariate analysis (OR of 1.44), but a multivariate analysis adjusting for age and sex made this association lose statistical significance (OR 1.19). The number of days of a hospital stay positively correlated with the days before a negative PCR after the first positive PCR (r = 0.60, p value < 0.0001).

Table 2. Risk for requiring mechanical ventilation among patients with overweight or obesity[1]

Normal (n=89)	Overweight (n=160)	Obesity (n=114)
Reference	1.47 (0.82-2.6)	1.7 (0.90-3.1)
Reference	1.63 (0.88-3.01)	1.97 (1.02-3.79)
Reference	0.67(0.29-1.53)	1.82 (0.94-3.53)

1 Values are Odds Ratio (95% CI) unless otherwise specified.
2 Model is multivariate analysis adjusted for age (continuous), sex (men or women), mean arterial pressure on admission (continuous), C reactive protein levels on admission (continuous), oxygen saturation on admission (continuous, percentage).

Table 3. Risk for requiring mechanical ventilation considering obesity as BMI >35 kg/m^2 [1]

Without obesity (n=318)	Obesity (n=37)
Reference	1.55 (0.77-3.08)
Reference	2.06 (0.99-4.28)
Reference	2.86 (1.09-7.5)

1 Values are Odds Ratio (95% CI) unless otherwise specified.
2 Model is multivariate analysis adjusted for age (continuous), sex (men or women), mean arterial pressure on admission (continuous), C reactive protein levels on admission (continuous), oxygen saturation on admission (continuous, percentage).

DISCUSSION

Obesity and COVID-19 coexist as pandemics in 2020, and obesity associates with increases in the severity, the need for mechanical ventilation, and mortality among these patients (15). In this study, we analyzed a cohort of 355 COVID-19 patients in Mexico City to compare the clinical characteristics and outcomes of lean, patients with overweight and obesity.

The prevalence of patients with overweight and obesity in our study was 31% and 45%, respectively, and

only 24% of patients had a normal BMI. A different cohort of COVID-19 patients in Mexico reported the prevalence of patients with overweight and obesity as 34.9% and 35.9%, respectively (16). The prevalence of patients with obesity and overweight in Mexico's general population, according to recent data, is 36.1% and 39.1%, respectively, which aligns with data in our cohort (7).

We divided our cohort into 3 groups according to WHO definitions: normal, overweight, and obesity (BMI > 30 kg/m^2). We found no significant association between patients with overweight or with obesity and a higher risk of requiring IMV. However, when conducting sensitivity analyses defining obesity as having a BMI > 35 kg/m^2, we found a higher risk of IMV (OR 2.86, 95% CI, 1.09-7.5). Overall, patients in the 3 groups received most treatments with similar frequencies. However, tocilizumab and lopinavir/ritonavir were more frequently prescribed for patients with overweight and obesity. The decision to initiate each treatment was based on the criteria of the attending physician, but none of these treatments impacted final outcomes (17,18).

Similar findings showed obesity as a risk factor for developing severe COVID-19, with the greatest impact in patients with a BMI ≥ 35 kg/m^2. In addition, 90% of this group of patients needed IMV (5). In a similar study in California, a BMI cutoff of > 40 kg/m^2 showed a greater risk of death after adjusting for obesity-related comorbidities (RR = 2.68, 95% CI, 1.43-5.04) (19).

These findings support that class II obesity (according to the WHO classification) constitutes a risk factor for needing IMV, but not for patients with overweight or those with class I obesity (20). Obesity-related comorbidities that might act as confounders (such as diabetes and hypertension) were similar in the three groups and did not associate with IMV in univariate analyses.

Patients with obesity have lower mortality in some chronic diseases. Similarly, patients with obesity have a lower acute respiratory distress syndrome associated mortality than does the general population (21). This phenomenon – the obesity paradox for chronic diseases – might also affect patients with COVID-19. Nevertheless, this paradox may merely reflect a selection bias (22). Early reports on COVID-19 suggested patients with overweigh and with obesity had an increased risk of complications, so it may be prudent to hospitalize these patients even if they present with mild disease (23). Another explanation for why overweight subjects and patients with lower grades of obesity have no higher risk for IMV is the concept of metabolically healthy obese patients. These patients have a preserved insulin sensitivity, a lower liver fat content, lower visceral fat mass, and normal adipose tissue function, conferring them a lower risk of mortality in some chronic diseases. This is further supported because obesity-related comorbidities, such as diabetes and hypertension, were similar in the three groups, as were glucose levels on admission but not for triglycerides and LDL-c levels (24).

For patients with class II or higher obesity, several mechanisms might increase their risk of mechanical ventilation. Adipose tissue is a proinflammatory tissue with an increased expression of cytokines, particularly adipokines like leptin, which associate with an increased inflammatory response, reduced ciliary clearance, and acute respiratory distress syndrome. A delayed immune response that impairs the response to infectious agents also appears in patients with obesity (21).

On the other hand, patients with obesity have excess body weight and poor pulmonary reserve, which alters pulmonary gas exchange and respiratory mechanics. This could necessitate early intubation and IMV due to an extremely rapid O2 desaturation, particularly in patients with higher degrees of obesity (25).

Our results show obesity could constitute an important risk factor for severe diseases requiring IMV, but only in patients with a BMI > 35 kg/m^2. Other studies found similar results but did not compare categories of patients of overweight or obesity using the conventional BMI cutoff > 30 kg/m^2. These findings suggest that only higher degrees of obesity increase the risk for IMV and worse outcomes with COVID-19. Not all patients with obesity and overweight might have the same risk, so they should be carefully evaluated to avoid preventive hospitalization when presenting a mild disease.

This study has some limitations, including its retrospective nature and its lack of non-hospitalized patients. However, it represents a tertiary care center's experience, which can support prospective studies around the country.

We conclude that patients with obesity, defined by the WHO as having a BMI > 30 kg/m^2, hospitalized for a SARS-CoV-2 infection had a similar risk for IMV compared to lean patients. The increased risk for IMV only appeared in patients with class II obesity.

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Incidence of thyroid diseases: Results from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

Isabela M. Benseñor[1]
https://orcid.org/0000-0002-6723-5678

José Augusto Sgarbi[2]
https://orcid.org/0000-0001-7187-984X

Carolina Castro Porto Silva Janovsky[1]
https://orcid.org/0000-0001-9383-7907

Bianca Almeida Pittito[3]
https://orcid.org/0000-0002-5907-5459

Maria de Fátima Haueisen Sander Diniz[4]
https://orcid.org/0000-0001-9146-5003

Maria da Conceição Chagas de Almeida[5]
https://orcid.org/0000-0002-4760-4157

Sheila Maria Alvim[6]
https://orcid.org/0000-0003-2080-9213

Sandhi M. Barreto[7]
https://orcid.org/0000-0001-7383-7811

Luana Giatti[7]
https://orcid.org/0000-0001-5454-2460

Bruce B. Duncan[8]
https://orcid.org/0000-0002-7491-2630

Maria Inês Schmidt[8]
https://orcid.org/0000-0002-3837-0731

Maria de Jesus M. Fonseca[9]
https://orcid.org/0000-0002-5319-5513

Rosane H. Griep[10]
https://orcid.org/0000-0002-6250-2036

Maria del Carmen B. Molina[11]
https://orcid.org/0000-0002-8614-988X

José Geraldo Mill[12]
https://orcid.org/0000-0002-0987-368X

Itamar de Souza Santos[1]
https://orcid.org/0000-0003-3212-8466

Alessandra C. Goulart[1]
https://orcid.org/0000-0003-1076-5210

Paulo A. Lotufo[1]
https://orcid.org/0000-0002-4856-8450

[1] Centro de Pesquisa Clínica e Epidemiológica, Universidade de São Paulo, São Paulo, SP, Brasil
[2] Divisão de Endocrinologia, Faculdade de Medicina de Marília, Marília, SP, Brasil
[3] Departamento de Medicina Preventiva, Universidade Federal de São Paulo, São Paulo, SP, Brasil
[4] Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brasil
[5] Instituto Gonçalo Moniz, Fundação Oswaldo Cruz – Fiocruz, Salvador, BA, Brasil
[6] Instituto de Saúde Coletiva, Universidade Federal da Bahia, Salvador, BA, Brasil
[7] Medicina Preventiva, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil
[8] Programa de Pós-Graduação em Epidemiologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil
[9] Departamento de Epidemiologia e Métodos Quantitativos, Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil
[10] Laboratório de Educação em Saúde e Meio Ambiente, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brasil
[11] Departamento de Nutrição, Universidade Federal do Espírito Santo, Vitória, ES, Brasil
[12] Departamento de Ciências Fisiológicas, Universidade Federal do Espírito Santo, Vitória, ES, Brasil

Correspondence to:
Isabela M. Benseñor
Centro de Pesquisa Clínica e Epidemiológica Hospital Universitário
Av. Lineu Prestes 2565
05508-000 – São Paulo, SP, Brasil

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ABSTRACT

Objective: To evaluate incidence of subclinical and overt hyperthyroidism and hypothyroidism. **Subjects and methods:** The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) is a prospective cohort study of 15,105 civil servants, examined at baseline and over a 4-year follow-up. This analysis included 9,705 participants with normal thyroid function at baseline, follow-up information about thyroid function and with no report of using drugs that may interfere in the thyroid function. Thyroid function was defined by TSH/FT4 levels or routine use of thyroid hormones/anti-thyroid medications. Annual and cumulative (over 4-year) incidence rates were presented as percentages (95% Confidence Intervals). **Results:** The incidence of all overt and subclinical thyroid disease was 6.7% (1.73%/year): 0.19% for overt hyperthyroidism (0.048%/year), 0.54% for subclinical hyperthyroidism (0.14%/year), 1.98% for overt hypothyroidism (0.51%/year), and 3.99% for subclinical hypothyroidism (1.03%/year). The incidence of all thyroid diseases was higher in women, when compared to men, with a low women:men ratio (1.36). For Blacks the highest incidence was for overt hyperthyroidism, while for Whites, the highest incidence was for overt hypothyroidism. However, the highest incidence of overt hyperthyroidism was detected in Asian descendants. The presence of antithyroperoxidase antibodies at baseline was associated with higher incidence of overt thyroid diseases. **Conclusions:** These results showed a high incidence of hypothyroidism, which is compatible with a country with a more-than-adequate iodine intake. The low women:men ratio of the incidence of thyroid dysfunction highlights the importance of the diagnosis of thyroid diseases among men in Brazil. Arch Endocrinol Metab. 2021;65(4):468-78

Keywords
Overt thyroid diseases; subclinical thyroid diseases; hyperthyroidism; hypothyroidism; incidence

INTRODUCTION

Thyroid dysfunction is a very common disease in the general population worldwide (1,2). Although there are a considerable number of prevalence studies, data on the incidence of thyroid diseases is still scarce. Few studies have evaluated the incidence of thyroid diseases worldwide, such as subclinical and overt hyperthyroidism and hypothyroidism (3,4). In Europe, a recent meta-analysis of seven studies reported an incidence of thyroid diseases of 259.12 (254.39-263.9) events per 100,000 per year: 226.2 (222.26-230.17) and 51 (49.23-52.88) events per 100,000 per year for hypothyroidism and hyperthyroidism, respectively (3). One study from Iran, one of the few low- and middle-income countries with information about incidence of thyroid diseases and known to be iodine-sufficient, reported an incidence of hypothyroidism of 3.3 in women and 2.1 in men per 1,000 persons/year, while the incidence of hyperthyroidism was found to be 3.8 in women and no cases in men per 1,000 persons/year after a 6-year follow-up (4).

Epidemiological studies on the incidence of thyroid diseases are very important, because many patients are asymptomatic or have reported unspecific symptoms, which can lead to a high rate of underdiagnosis (1,2,4). Additionally, the incidence of thyroid disorders is related to the availability of iodine (5,6), selenium and other trace elements (7), chemical contaminants (8), frequency of autoimmune thyroid diseases (9), and genetic risk factors. (10) Incidence also varies widely in populations according to the area of residence. (5-7) Finally, as recently proposed, subclinical thyroid diseases can be considered a non-classical risk factor for coronary heart disease (11), being associated with all-cause mortality (12-14) and cardiovascular mortality (13,15).

In Brazil, some information can be found regarding the prevalence of thyroid diseases in population-based (16,17) and large epidemiological studies (18). The available data indicate rates within the highest prevalence of hypo- and hyperthyroidism in the world (19). However, to the best of our knowledge, no large study on the incidence of thyroid diseases in Brazil has been conducted to date. Therefore, the objective of this analysis is to evaluate the incidence of all overt and subclinical hypothyroidism and hyperthyroidism using data from the baseline and over a 4-year follow-up of the Brazilian Longitudinal studies of Adult Health (ELSA-Brasil).

SUBJECTS AND METHODS

The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) is a prospective cohort study of civil servants, 35 to 74 years of age, in six cities in Brazil, examined at baseline (2008-2010) and after a 4-year follow-up (2012-2014). Although the study is focused on cardiovascular diseases and diabetes, together with associated factors, it also includes information about non-classical risk factors for cardiovascular disease, such as the subclinical thyroid function (20,21).

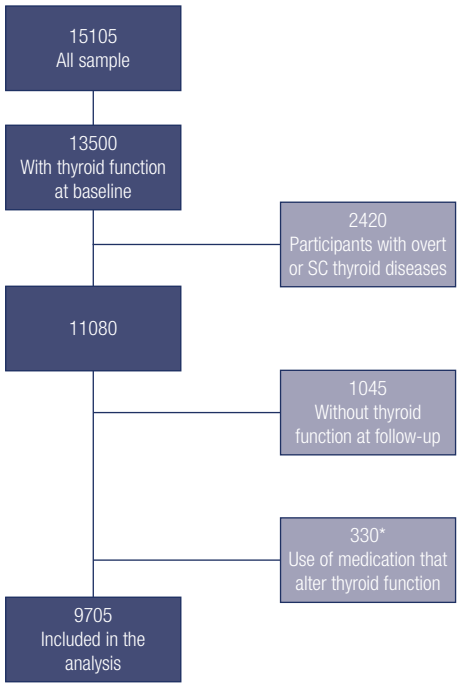
Briefly, all 15,105 participants are civil servants of six teaching and research institutions located in six different cities in Brazil: Salvador (*Universidade Federal da Bahia* – UFBA, 2029 participants), Vitória (*Universidade Federal do Espírito Santo*, 1055 participants), Belo Horizonte (*Universidade Federal de Minas Gerais*, 3115 participants), Rio de Janeiro (*Fundação Oswaldo Cruz*, 1784 participants), São Paulo (*Universidade de São Paulo* – USP, 5061 participants), and Porto Alegre (*Universidade Federal do Rio Grande do Sul*, 2061 participants). There are no important differences among sites. All participants are civil servants with higher education attainment and average monthly family net income compared to general population in Brazil. ELSA-Brasil sample is similar to people living in metropolitan areas in Brazil. Although the higher education attainment and average income, in all 6 centers, sample was selected according to 3 categories: non-skilled, technical and faculties with a clear socioeconomic gradient among them.

The sample size estimation was calculated to allow sex-specific analyses. The ELSA-Brasil protocol was approved at each of the six study centers by the local Institutional Review Board, which addresses research in human participants. All participants provided a signed informed consent (CAAE Number at Plataforma Brasil: 08109612.7.1001.0076). The second evaluation of the participants was performed between 2012 and 2014. In both examinations at baseline and follow-up each participant was interviewed and examined in the study research centers, following standard protocols developed for the study. Trained study staff conducted the interviews and examinations, following strict quality control procedures as previously described (22,23).

Participants

This analysis included all participants with information about thyroid function at baseline and over the 4-year

follow-up (Figure 1). The main reasons for not having data on thyroid function in follow-up analyses were death or retirement with change of place of residence outside the metropolitan area of the Research Center and not being available to come to the clinical center for examination.



* List of medications used by participants that alter thyroid function and were excluded from the analysis: amiodarone, biotin, carbamazepine, carbidopa, divalproex sodium, furosemide, haloperidol, heparina, levodopa, lithium, metoclopramide, oxcarbazepine, phenytoin, propranolol, primidone, rifampicin, sodium valproate, valproic acid.

Figure 1. Participants included in the analysis.

Definition of thyroid function

All tests were done in the Central Laboratory localized at *Hospital Universitário*, USP, São Paulo, Brazil. Blood samples were drawn in each center, centrifuged to obtain serum for biochemistry and to determine hormone levels and aliquoted in cryotubes at -80ºC. Each month, the samples were transported to São Paulo and the tests were done in the Central Laboratory. The Research Center of São Paulo was responsible for a centralized training of all local laboratory teams in São Paulo as well as under strict quality control of all tasks (24).

Thyrothropin (TSH) and free-thyroxine (FT4) levels were determined by a third-generation immunoenzymatic assay (Roche Diagnostics, Manheim, Germany). Thyroid dysfunction was defined by TSH and FT4 levels or by the routine use of thyroid hormones or anti-thyroid medications, such as propylthiouracil or thiamazole. Cut-off for normal TSH was 0.4-4.0 mIU/L and for normal FT4 was 0.93–1.7 ng/dL. Levels of anti-thyroperoxidase antibodies (TPOAb) were measured by electrochemiluminescence (Roche Diagnostics, Mannheim, Germany) and were considered positive when ≥34 IU/mL and negative as <34 IU/mL.

Based on TSH and FT4 levels and the use of medications to treat thyroid disorders, participants were categorized into the five following groups: (1) overt hyperthyroidism (low TSH, high FT4, or use of medications to treat hyperthyroidism), (2) subclinical hyperthyroidism (low serum TSH, normal FT4, and no use of thyroid drugs), (3) euthyroidism (normal TSH without use of thyroid medication), (4) subclinical hypothyroidism (high TSH, normal FT4 levels, and no use of thyroid drugs), and (5) overt hypothyroidism (high TSH, low FT4, or use of levothyroxine).

Other baseline variables

Fasting plasma glucose was measured using a hexokinase method. Total cholesterol and HDL-cholesterol were measured, using enzymatic colorimetric assay (ADVIA Chemistry). Triglycerides were also measured by using enzymatic colorimetric assay (glycerol phosphate peroxidase) (ADVIA Chemistry), while LDL cholesterol was calculated by applying the Friedewald equation (24). The study questionnaire addressed sociodemographic factors, including age (continuous or categorical, such as 35 to 44, 45 to 54, 55 to 64, and 65 to 74 years of age) and sex, and the level of formal education (less than high-school, high-school and some college, and at least complete college or more. The study evaluated self-reported race/skin color as a social construct using the same question used in the Brazilian CENSUS (IBGE): "The Brazilian census (IBGE) describes people's color or race as "Black", "Brown", "White", "Asian descendant" or "Brazilian indigenous". If you were to answer the IBGE census today, how would you describe your own color or race?" The following response options were given: Black, Brown, White, Asian descendant, and Brazilian indigenous. All participants were asked about their use of prescription and nonprescription drugs and were requested to present their prescriptions, packages,

package inserts and/or blister packs of the medications used in the last two weeks. All proceedings were performed in the same way at baseline and follow-up.

Statistical analysis

The incidence of thyroid disease rates is expressed as percentage per year or as a cumulative incidence over a 4-year follow-up with respective 95% Confidence Intervals (95% CI). Categorical variables are reported as proportions and compared using the Chi-square test. Continuous variables with normal distribution are reported as means (Standard deviation, ±SD) and compared by analysis of variance (ANOVA), while those with non-normal distribution are presented as medians (Interquartile range, IQR) compared by means of the Kruskal-Wallis test. For showing differences among categories of each variable it was used Bonferroni post hoc test for categorical and continuous nonparametric variables.

Analysis were conducted using the Statistical Package for Social Sciences (SPSS) version 25. Confidence Intervals were calculated using the software R version 3.5.3 (R Core Team, Vienna, Austria).

RESULTS

After exclusions (Figure 1), 9,705 participants (median age, 50 (IQR, 45-57); 51.9% of women) with normal thyroid function at baseline examination that also have data about thyroid function over the 4-year follow-up were included in the incidence analysis. Over a 4-year follow-up, of the 9,705 participants, 9,079 remained with normal thyroid function (euthyroid), 17 developed incident overt hyperthyroidism, 49 incident subclinical hyperthyroidism, 377 incident subclinical hypothyroidism, and 183 incident overt hypothyroidism. Table 1 shows baseline sociodemographic and clinical characteristics of the 9,705 participants according to the incidence of thyroid disorders over a 4-year follow-up. Most cases of thyroid diseases were found in womencompared to men ($P < 0.0001$). Median age increases from overt hyperthyroidism to overt hypothyroidism. Frequency of thyroid diseases were different according to age-strata and race (respectively $P < 0.0001$ and $P = 0.01$).

Table 2 shows an annual incidence of thyroid diseases according to sex, age-strata, and self-reported race. The incidence of overt hyperthyroidism was similar for men and women. The incidence of subclinical hyperthyroidism and overt hypothyroidism was higher in women when compared to men. However, the incidence of subclinical hypothyroidism is discretely higher in men when compared to women (1.11% in men vs. 0.99% in women but the difference was not statistically significant, $P = 0.38$). Considering all dysfunctions together, the women:men ratio for thyroid diseases was 1.36.

Figure 2 shows the cumulative incidence of thyroid diseases according to age-strata in the entire sample by sex. The results showed a cumulative incidence of thyroid disease of 6.7% (1.73% per year): 0.19% for overt hyperthyroidism (0.048% per year), 0.54% for subclinical hyperthyroidism (0.14% per year), 1.98% for overt hypothyroidism (0.51% per year), and 3.99% for subclinical hypothyroidism (1.03% per year). In the entire sample, the incidence of hypothyroidism is higher than for hyperthyroidism. The incidence of hypothyroidism is also higher in the older-age strata for entire sample, including both men and women while incidence of hyperthyroidism was higher in the younger age-strata. As expected, the incidence of thyroid diseases is higher in women when compared to men. However, the women:men ratio for overt hyperthyroidism, subclinical hyperthyroidism, and overt hypothyroidism and subclinical hypothyroidism were, respectively, 1.1, 2.3, 0.9 e 3.2.

Of the 17 incident cases of hyperthyroidism, 9 (52.9%) were identified based on TSH and FT4 levels and 8 (47.1%) were identified by use of medication. Of them, 9 (52.9%) were women and 7 of them (77.8%) were under treatment. Of the 8 men with overt hyperthyroidism, only 1 (12.5%) were under treatment. Mean, median and range of participants treated with thiamazole were respectively 7.5 (±1.0), 7.5 (5-10), and range of 5 to 10 mg. Of the 183 incident cases of hypothyroidism, 49 (26.8%) were identified based on TSH and FT4 levels and 134 (73.2%) were identified by use of medication. Of them, 142 (77.6%) were women and 113 (79.6) of them where under treatment. However, of the 41 men with overt hypothyroidism, only 21 (51.2%) were under treatment with levothyroxine. ($P < 0.0001$). Mean, median and range of levothyroxine in participants treated with levothyroxine were respectively 90 mcg (±14.7), 75 mcg (38-100) with a range from 25 to 143 mcg.

The incidence of overt hyperthyroidism is the highest in participants who self-reported themselves Asians (0.10%, 95% CI, 0.2-1.1) followed by Blacks (0.067%, 95% CI, 0-0.43) Mixed (0.05%, 95% CI, 0-0.27) and Whites (0.04%, 95% CI 0.01-0.17). The

Table 1. General characteristics of all 9,705 subjects according to the presence or not of thyroid function over a 4-year follow-up (2012-2014)

	Overt hyperthyroidism N = 17	Subclinical hyperthyroidism N = 49	Euthyroidism N = 9,079	Subclinical hypothyroidism N = 377	Overt hypothyroidism N = 183	All N = 9705	P
Age (years)**	49 (43.5-51)	52 (49-58)	50 (44-57)*†	53 (47-59)†	53 (46-60)*	50 (45-57)	<0.0001
Age-strata at baseline							<0.0001
35-44	7 (41.2)*	8 (16.3)†	2766 (30.5)*†‡	80 (21.2)*†‡	41 (22.4)‡	2411 (24.8)	
45-54	9 (52.9)	20 (40.8)	3611 (34.8)	148 (39.3)	73 (39.9)	3953 (40.7)	
55-64	1 (5.9)	17 (34.7)	2093 (23.1)	112 (29.7)	45 (24.6)	2513 (25.9)	
65-74	0(0)*	4 (8.2)†	609 (60.3)*†‡	37 (9.8)‡	24 (13.1)*†‡	828 (8.6)	
Women (%)	9 (52.9)*	35 (51.4)*	4665 (51.4)*	185 (49.1)*	142 (77.6)*	5036 (51.9)	<0.0001
Race (%)							0.01
White	7 (41.2)*	30 (61.2)†	4552 (50.7)*†‡	230 (61.2)*†‡	109 (59.9)‡	4928 (51.4)	
Mixed	5 (29.4)	11 (22.4)	2554 (28.5)	83 (22.1)	41 (22.5)	2694 (28.1)	
Black	4 (23.5)	7 (14.3)	1525 (17)	54 (14.4)	21 (11.5)	1611 (16.8)	
Asian	1 (5.9)	1 (2)	242 (2.7)	5 (1.3)	9 (4.9)	258 (2.7)	
Indigenous	0	0	97 (1.1)	4 (1.1)	2 (1.1)	103 (1.1)	
Education (%)							0.74
Less than high-school	3 (17.6)	4 (8.2)	1087 (12)	47 (12.5)	21 (11.5)	1162 (12)	
High-school and some College	7 (41.2)	20 (40.8)	3249 (35.8)	132 (35)	55 (30.1)	3463 (35.7)	
Complete College or more	7 (41.2)	25 (51)	4743 (52.2)	198 (52.5)	107 (58.5)	5080 (52.3)	
Thyroid stimulating hormone (IU/ml)**	0.01 (0.01-1.63)*#¥▪	0.28 (0.10-0.34)†‡	1.79 (1.30-2.40)*# ♦α	4.64 (4.24-5.22)‡▪αβ	2.53 (1.04-4.55)†¥♦β	1.84 (1.31-2.52)	<0.0001
Free-thyroxine (ng/ml)**	2.08 (1.28-2.90)†‡#	1.24 (1.12-1.36)*	1.18 (1.08-1.28)‡	1.15 (1.08-1.25)*†	1.24 (0.90-1.56)#	1.18 (1.08-1.29)	<0.0001
TPOAbs (%)**	17.1 (10.2-126.7)‡	14.61 (11.42-30.1)†	11.69 (11.15-14.86)*†‡	11.5 (8.75-16.5)#	13.19 (9.68-63.13)*#	11.19 (8.71-15.06)	<0.0001

**Median (Interquartile Range); For age: *Euthyroidism ≠ overt hypothyroidism; †euthyroidism ≠ subclinical hypothyroidism; For age-strata 35-44 years of age, *overt hyperthyroidism, †subclinical hyperthyroidism and ‡overt hypothyroidism ≠ from euthyroidism and subclinical hypothyroidism; for age-strata 65-74, *overt hyperthyroidism, †subclinical hyperthyroidism and ‡subclinical hypothyroidism ≠ euthyroidism and overt hypothyroidism. For *women and for †men, each category is ≠ all others; For self-reported skin color White *overt hyperthyroidism, †subclinical hyperthyroidism and ‡overt hypothyroidism ≠ euthyroidism;and *†‡subclinical hypothyroidism. For TSH: *Overt hyperthyroidism ≠ euthyroidism (P <0.0001); †Subclinical hyperthyroidism ≠ overt hypothyroidism (P<0.0001); ‡Subclinical hyperthyroidism ≠ subclinical hypothyroidism (P <0.0001); #Overt hyperthyroidism ≠euthyroidism (P = 0.002); ¥Overt hyperthyroidism ≠overt hypothyroidism; ▪Overt hyperthyroidism ≠subclinical ypothyroidism (P <0.0001); ♦Overt hypothyroidism ≠ euthyroidism (P <0.0001); αSubclinical hypothyroidism ≠ euthyroidism (P <0.0001); βOvert hypothyroidism ≠ subclinical hypothyroidism; For FT4: *Subclinical hyperthyroidism ≠ subclinical hypothyroidism (P = 0.045); †Overt hyperthyroidism ≠ subclinical hypothyroidism (<0.0001); ‡Overt hyperthyroidism ≠ euthyroidism (P <0.0001); #Overt hyperthyroidism ≠ overt hypothyroidism, (P = 0.002); For TPOAb: *Overt hypothyroidism ≠ euthyroidism (P <0.0001); †Subclinical hyperthyroidism ≠ euthyroidism (P <0.0001); ‡Overt hyperthyroidism ≠ euthyroidism (P = 0.046); #Overt hypothyroidism ≠ subclinical hypothyroidism (P = 0.01).

incidence of overt hypothyroidism was higher in Whites 0.61%, 95% CI, 0.42-0.89), followed by Asians (0.52, 95% CI, 0.05-2.76), and Indigenous (0.52%, 95% CI,0.05-2.76) with the lower and similar incidences for Mixed (0.40%, 95% CI, 0.21-0.76) and Blacks (0.35%, 95% CI, 0.13-0.84). Participants who self-reported themselves as Mixed race presented an incidence of overt hyperthyroidism and overt hypothyroidism that is intermediary between Whites and Blacks (Table 2). Figure 3 shows the cumulative incidence of thyroid dysfunction according to self-reported race.

Figure 4 shows the incidence of thyroid diseases over a 4-year follow-up according to the presence or not of TPOAb at baseline. Incidence of thyroid diseases is always higher in participants with positive TPOAb, when compared to participants with no TPOAb, at baseline. The ratio of thyroid diseases in participants with positive TPOAb, when compared to participants with negative TPOAb, at baseline for overt hyperthyroidism, subclinical hyperthyroidism, subclinical hypothyroidism, and overt hypothyroidism were 5.6, 2.7, 2.3, and 5.2, respectively.

Table 2. Annual incidence (95% Confidence Interval – 95% CI) of thyroid diseases expressed in percentages according to sex, age-strata at baseline and self-reported race

	Overt hyperthyroidism N = 17	Subclinical hyperthyroidism N = 49	Subclinical hypothyroidism N = 377	Overt hypothyroidism N = 183	All thyroid diseases	P
Sex (%)						<0.0001
Women	0.052* 0.01-0.19	0.19* 0.09-0.38	0.99* 0.74-1.32	0.76* 0.54-1.06	1.99 0.12-14.6	
Men	0.047† 0.01-0.18	0.082† 0.02-0.24	1.11† 0.83-1.47	0.24† 0.13-0.45	1,48 (0-11.93)	
All	0.048 0.02- 0.13	0.14 0.08-0.25	1.03 0.84-1.26	0.51 0.38-0.68	1.73 1.48- 2.01	
Age-strata (years)						
35-44	0.056* 0.01-0.30	0.09† 0.02-0.35	0.73*†‡ 0.44-1.19	0.41‡ 0.21-0.79		<0.0001
45-54	0.07 0.02-0.24	0.12 0.04-0.31	0.98 0.70-1.36	0.47 0.29-0.76		
55-64	0.02 0-0.24	0.21 0.08-0.52	1.25 0.86-1.80	0.55 0.31-0.96		
65-74	0* 0-0.64	0.17† 0.02-0.92	1.50‡ 0.81-1.09	0.90*†‡ 0.39-1.93		
Self-reported race (%)						P = 0.01
White	0.04* 0.01-0.17	0.17† 0.08-0.35	1.26*†‡ 0.97-1.63	0.61‡ 0.42-0.89		
Mixed	0.050 0-0.27	0.11 0.03-0.36	0.80 0.51-1.25	0.40 0.21-0.76		
Black	0.067 0-0.43	0.12 0.02-0.51	0.87 0.49-1.50	0.35 0.13-0.84		
Asian	0.10 0-2.11	0.10 0-2.11	0.91 0.18-3.34	0.52 0.05-2.76		
Indigenous	0 0-4.75	0 0-4.75	1.01 0.06-6.21	0.52 0-5.52		

Annual incidence expressed in percentages. For *women and †men: each groups is ≠ from the others. For age-strata 35-45, *overt hyperthyroidism, †subclinical hyperthyroidism and ‡overt hypothyroidism are ≠ from euthyroidism and *†‡subclinical hypothyroidism; for age-strata 65-74, *overt hyperthyroidism, †subclinical hyperthyroidism and ‡subclinical hypothyroidism ≠ euthyroidism and *†‡overt hypothyroidism;

For self-reported skin color: white *overt hyperthyroidism, †subclinical hyperthyroidism and ‡overt hypothyroidism ≠ euthyroidism and subclinical *†‡subclinical hypothyroidism.

DISCUSSION

The present study showed, for the first time, a high annual and cumulative incidence of new overt and subclinical hyperthyroidism and hypothyroidism in a large Brazilian cohort after a 4-year follow-up. The results highlighted a higher incidence of hypothyroidism in the country, but a similar incidence of hyperthyroidism compared to other countries worldwide. Our data also presented a lower women:men ratio for thyroid diseases compared to other classical studies that evaluate thyroid function. Incidence of overt hypothyroidism, but not of overt hyperthyroidism increased with ageing. Incidence of thyroid diseases was higher in participants with positive TPOAb at baseline compared to other participants.

The overall incidence of thyroid dysfunctions was higher in women, when compared to men, as demonstrated by the women:men ratio of 1.36, but lower than the ratios reported by classical studies on the incidence or prevalence of thyroid diseases (13,25). Frequency of treatment of overt thyroid diseases is higher in women compared to men. The presence of TPOAb antibodies was associated with a higher incidence of all thyroid diseases. Regarding treatment, more women than men are under treatment for thyroid diseases even considering a sample with more access to health care compared to the general population in Brazil. This low frequency of treatment in men confirmed a previous study in a population-based sample of older men and women (16).

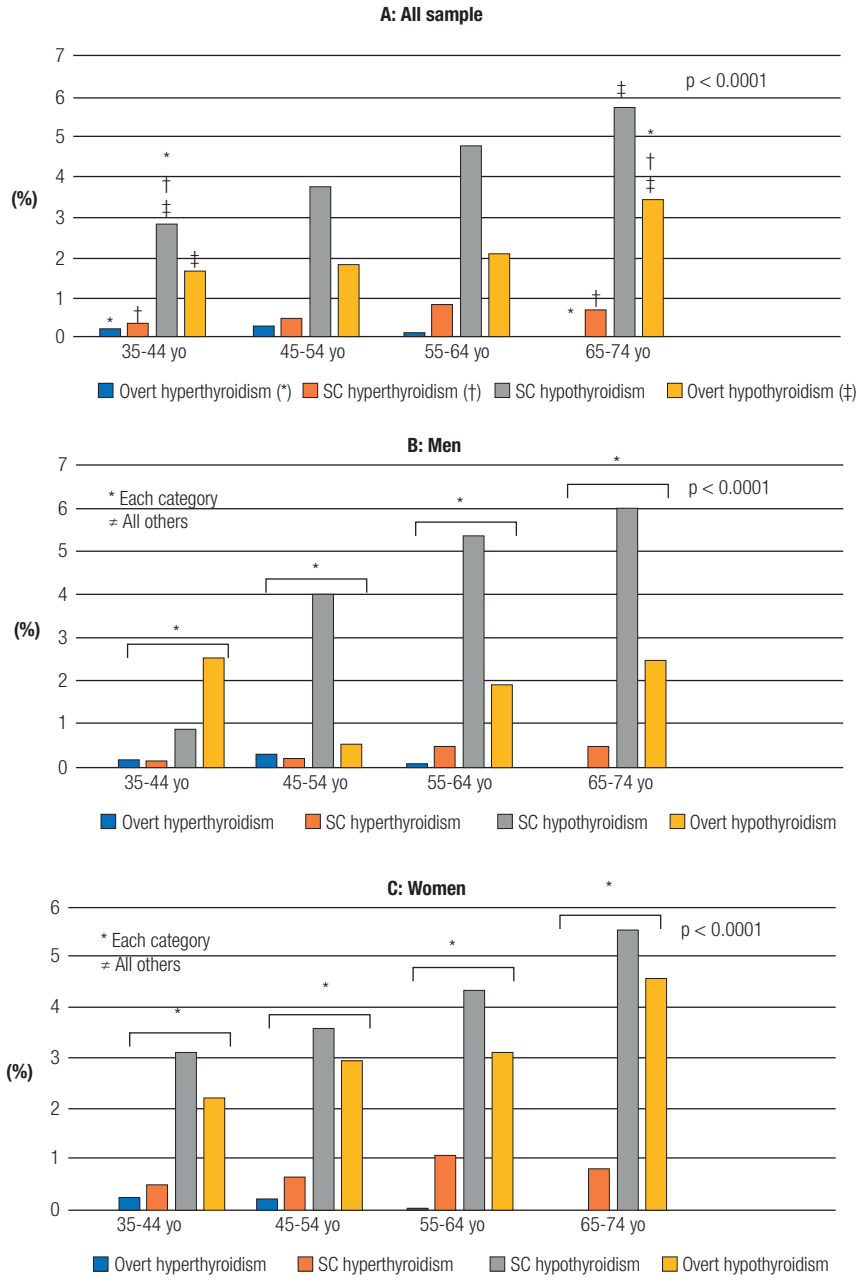


Figure 2. Cumulative incidence of thyroid diseases according to age-strata (yo = years of age).

A meta-analysis of seven studies in Europe reported an incidence of 226.2 (222.26-230.17 per 100,000) for hypothyroidism (3). These numbers were lower than Brazilian data if our results were expressed per 100,000 per year (for overt hypothyroidism in ELSA-Brasil of 511.87/100,000). Another important point is that, although we showed a higher incidence in women when compared to men, the women:men ratio for overt hypothyroidism is 3.2 which is lower than the women:men ratio in this European meta-analysis of 5.1 for overt hypothyroidism (3). The low women:men ratio was previously reported in the prevalence of thyroid diseases at the ELSA-Brasil baseline (18) as well as in a population-based sample of older adults with a diagnosis of thyroid diseases in the city of São Paulo (16).

For overt hyperthyroidism, the European meta-analysis reported an incidence of 51 (49.23-52.88) for hyperthyroidism, which is similar to the incidence

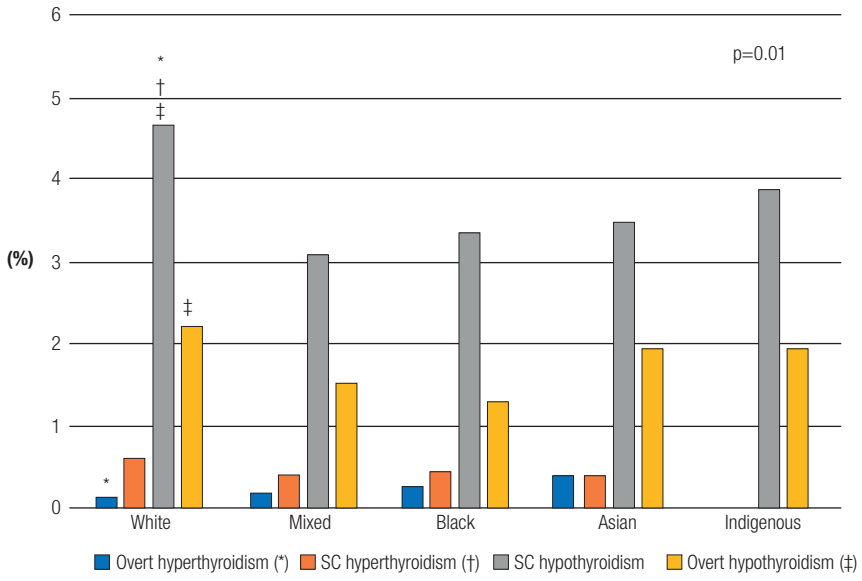


Figure 3. Cumulative incidence of hyperthyrodism and hypothyroidism according to self-reported race.

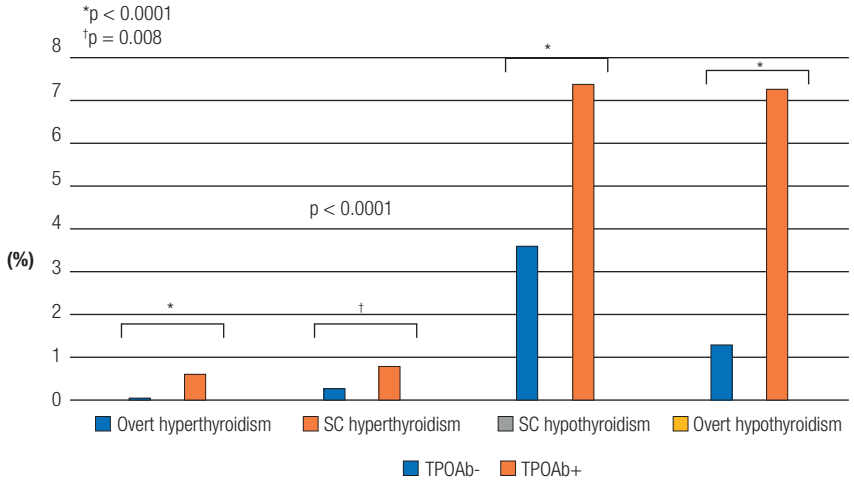


Figure 4. Incidence of overt and subclinical hyperthyroidism and hypothyroidism according to the presence or not of TPO antibodies (TPOAb) at baseline.

in ELSA-Brasil if it were expressed by 100,000 (48.42/100,000). Once again, the women:men ratio for overt hyperthyroidism of 1.1 was lower than the women:men ratio in the meta-analysis of 5.1.

Gopinath and cols. evaluated the 5-year incidence of thyroid dysfunction in a sample of older adults (≥ 55 years) in Australia, reporting an incidence of all thyroid diseases of 4.7% compared to 6.7% in Brazil. (26) As the incidence of hyperthyroidism was similar in both countries, the difference in the percentage of thyroid diseases is related to a higher incidence of hypothyroidism in Brazil compared to Australia, even considering that the median age of our sample is younger than that in Gopinath's study (mean age in Gopinath's study: 67.6 ± 7.6; our study: 51.2 ± 8.9) and our follow-up is shorter (4 years compared to 5 years in the Australian study. The results from the present study regarding high incidence of hypothyroidism and a similar incidence of hyperthyroidism when compared to some other countries are in accordance with previous studies that showed a lower incidence of hyperthyroidism and a higher incidence of hypothyroidism in places with higher levels of iodine intake (6,27,28), such as the case of Brazil. The presence of goiter was very high in the past and decreased after the iodination of salt, which began in 1953. Since the beginning of the twenty-

first century, the discussion about salt iodination in Brazil has become a matter of intense debate. A meta-analysis of seven studies from the Southeast region of Brazil showed great heterogeneity among studies, with no available data from other Brazilian regions (29). More recently, results from the PNAISAL (30) (National Research to Evaluate the Impact of Salt Iodination), a national research in Brazil including 18,978 schoolchildren from all regions of the country, conducted to evaluate the iodine nutritional status in the Brazilian population classified Brazil as a country with a more-than-adequate iodine intake.

Incidence of hypothyroidism increased with age as expected. However, not in the same pace as studies in countries with a high prevalence of olders. (25,26,28,31) Our data is in agreement with data from another low- and middle-income country that also showed a moderate increase of hypothyroidism with age (4). For overt hyperthyroidism, our data showed a higher incidence in the younger age-strata. As the number of participants in the younger age-strata is greater than in the older age-strata and the incidence of overt hyperthyroidism is lower than for hypothyroidism, in a sample of middle-aged adults, it is more difficult to show the increase with ageing. Our data is similar to data from another low- and middle-income country that also did not show an increase in the incidence of overt hyperthyroidism with ageing (4). In addition, Brazil has still a higher early mortality especially for cardiovascular diseases compared to high-income which difficult to show increasing trends in old-age for several variables.

The ELSA-Brasil study examined a highly admixed population, with a considerable proportion of participants that classified themselves as Mixed race. Although race/skin color in ELSA-Brasil was measured using the question of the Brazilian census as a social construct, results of higher incidence of hypothyroidism in whites and hyperthyroidism in blacks are in accord with previous studies that measure race/skin-color in different ways (32-34). However, it is important to notice that the highest incidence of overt hyperthyroidism was detected in Japanese descendants and for hypothyroidism Asians was in the second place after Whites. This was in agreement with a previous study of Sgarbi et al. with Brazilian Japanese (14) but not with the prevalence/incidence of thyroid diseases in Japan (35,36) that is much lower, suggesting the influence of environment and lifestyle more than genetic inheritance to explain results in Japanese descendants in Brazil. Results for the incidence of overt hypothyroidism in the Indigenous category have to be interpreted with caution because of the small number in the sample.

Our results showed that euthyroid individuals with positive TPOAb presented a higher incidence of overt and subclinical hyperthyroidism and hypothyroidism than TPOAb negative participants. Our results are in accord with previous published studies. (37-40) For incident overt hypothyroidism and hyperthyroidism the risk of participants TPOAb positive is 5.2 and 5.6 times higher compared to participants with negative TPOAb respectively. The ratio of conversion for overt thyroid diseases were higher than for subclinical thyroid diseases. A recent analysis about the prevalence of TPOAb in Brazil using data from ELSA-Brasil study showed values in accordance with a country with adequate iodine intake (41). It would be interesting to monitor TPOAb levels and incidence of thyroid diseases using data from the third data collection over a longer follow-up.

Our study has some limitations. ELSA-Brasil is not a population-based study. However, it is a multicentric cohort study in six different cities located in three different regions of the country: South, Southeast, and Northeast. The study includes a sample with higher education and higher average monthly net family income compared with the general population of Brazil. Although these differences between ELSA-Brasil participants and general population in Brazil, the social and ethnic diversity of the cohort is similar to the heterogeneous populations of mostly low- and middle-income people living in large cities in Brazil. This suggests that our external validity may extend to urban centers with similar characteristics both inside and outside of Brazil. Furthermore, there are several similarities in the prevalence of selected behavioral risk factors and chronic conditions, as these have been assessed with similar procedures in the ELSA-Brasil and in the Surveillance System of Risk and Protection Factors for Chronic Diseases by Telephone Survey (VIGITEL), an annually performed telephone-based behavioral risk factor survey, producing representative data for adults living in Brazil's 27 state capitals and the Federal District (42). Overt hypothyroidism was defined based on TSH and FT4 levels and the use of levothyroxine. However, levels of TSH and FT4 were measured only one time, not considering the great

intra-individual variability of TSH values and some occasionally variations of TSH levels may be interpreted erroneously as permanent disease. At baseline of the ELSA-Brasil, a high use of levothyroxine was reported in the sample, especially in women with a higher average monthly family net income, when compared to others. Therefore, both problems may contribute to some degree of misclassification, especially regarding subclinical and overt hypothyroidism in women in the present analysis.

This study also has some strengths. Thyroid function at baseline and after a 4-year follow-up were measured, which allowed us to determine the incidence of thyroid diseases in Brazil for the first time in a large and multicentric sample. Although the higher frequency of women, the study included a similar number of men. Study protocols were exactly the same for all centers, and data collection was performed under strict quality control. These results in a highly admixed population bring original information about the incidence of thyroid diseases in participants that self-reported themselves as Mixed, a group that, except for a previous study in Brazil (17), had never been analyzed in other studies.

In conclusion, results from ELSA-Brasil showed that the high incidence of hypothyroidism may well be compatible with a country with a more-than-adequate iodine intake. Incident data also showed a low women:men ratio for thyroid diseases and a lower proportion of treatment in men with overt thyroid diseases compared to women. Both data highlight the importance of the diagnosis and treatment of thyroid diseases among men in Brazil.

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Galanin and glypican-4 levels depending on metabolic and cardiovascular risk factors in patients with polycystic ovary syndrome

Sunduz Ozlem ALTINKAYA[1]
https://orcid.org/0000-0002-0991-7443

[1] Adnan Menderes University, Faculty of Medicine, Aydin, Turkey

ABSTRACT

Objective: Galanin is a neuropeptide which has effects not only on metabolic syndrome but also on reproduction. Glypican-4 is an adipokine associated with insulin sensitivity by interacting directly with the insulin receptor. This study evaluated serum concentrations of galanin and glypican-4 in relation with the hormonal profile as well as metabolic and cardiovascular risk factors in patients with and without polycystic ovary syndrome (PCOS). **Subjects and methods:** A total of 44 women with PCOS and 44 age-matched controls were eligible. Hirsutism scores, hormonal profile, metabolic and cardiovascular risk factors as well as galanin and glypican-4 levels were evaluated in each subject. **Results:** Women with PCOS exhibited lower levels of galanin (20.2 pg/mL versus 26.4 pg/mL, $p = 0.002$) and higher concentrations of glypican-4 (3.1 ng/mL versus 2.6 ng/mL, $p < 0.001$) than controls. Both adipokines were correlated positively with body mass index (BMI), insulin, triglyceride and Homeostasis Model Assessment (HOMA) index; glypican-4 also showed positive correlations with fasting blood glucose, free testosterone, modified Ferriman-Gallwey scores ($p < 0.05$). Multiple Linear Regression analyses showed that PCOS and BMI were the best predictors affecting galanin levels with a decreasing and increasing effect respectively; however BMI was the best predictor affecting glypican-4 levels with an increasing effect ($p < 0.001$). **Conclusion:** Galanin levels were lower and glypican-4 levels were higher in women with PCOS than controls. Further studies are needed to determine whether these adipokines could be used as additional markers for insulin sensitivity and lipid profile and whether they might play a role in the pathogenesis of PCOS, in which metabolic cardiovascular risks are increased. Arch Endocrinol Metab. 2021;65(4):479-87

Keywords

Polycystic ovary syndrome; galanin; glypican-4

Correspondence to:
Sunduz Ozlem ALTINKAYA
Adnan Menderes Üniversitesi
Tip Fakültesi, Aydin, Turkey
altinkayaozlem@yahoo.com

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder and the cause of ovulatory dysfunction in women of reproductive age. It occurs at a frequency of 5–15%, depending on the diagnostic criteria applied (1,2). In spite of the variable clinical expression, the syndrome is classically described by ovulatory dysfunction, hyperandrogenism and polycystic ovaries (1). Unfortunately, the multisystem effects of the syndrome span from systemic metabolic disturbances to reproductive dysfunction, in addition to long-term cardiovascular event and cancer risk (1,2). The more severe PCOS phenotypes are associated with further emphasis of cardiovascular disease risk (3). Both obese and lean women with PCOS have various levels of insulin resistance. Given the interrelation with insulin resistance, all women diagnosed with PCOS need to be evaluated for the risk of metabolic syndrome and associated diseases, such as type 2 diabetes, hypertension, hyperlipidaemia, and the possible risk of some clinical emergency events, including acute myocardial infarction and stroke (4).

Galanin, a member of the galanin peptide family (galanin, galanin-like peptide, alarin), is a 29-amino-acid neuropeptide, and involved in regulating appetite, obesity, insulin resistance, hypertension and metabolism (5). It was first isolated from porcine intestine by Tatemoto and cols. (6) nearly 35 years ago. This peptide is not only found in central and peripheral nervous systems but also in the human carotid body, skeletal and heart muscle, adipose tissue and pancreas (7,8). Galanin has effects on metabolic syndrome with regard to food consumption, preference for a high-fat diet, elevation in the probability of obesity and dyslipidaemia and decreased insulin resistance and blood pressure to relieve the risk for type 2 diabetes

mellitus and hypertension (5). Galanin is a crucial neuropeptide which supports glucose transport via GLUT-4, an insulin-regulated glucose transporter. The overall effect of galanin on the metabolic syndrome may be summarised briefly into two categories. First, it causes an increase in GLUT-4 translocation to promote glucose intake, therefore elevating insulin sensitivity. Second, it decreases insulin secretion from pancreatic islet cells (5). It may interact with other peptides that play roles in regulating appetite, such as neuropeptide-Y and leptin, and this orchestration could play a role in the pathogenesis of the metabolic syndrome (9). In addition, galanin is a target agent for sex steroids, serving as molecular motifs integrating the control of metabolism as well as reproduction (10). It is released in a pulsatile manner, similar to the gonadotrophin-releasing hormone (GnRH) (10), and stimulates the secretion of luteinizing hormone (LH) in the porcine; in mice, it inhibits LH secretion (11,12).

Glypican-4 was first discovered in 1995 in the kidneys and developing brain tissue of mice by Watanabe and cols. (13). Gesta and cols. (14) demonstrated that the genetic expression of glypican-4 differs in visceral and subcutaneous fat tissues. The authors found that lower subcutaneous and higher visceral adipose tissue expression of glypican-4 appear to be associated with an increased waist-hip ratio (WHR) and body mass index (BMI), indicating higher risks for metabolic and cardiovascular complications (14). Recently, glypican-4 has been identified as a novel adipokine, which is a cell surface proteoglycan and enhances insulin receptor signalling and adipocyte differentiation by interacting directly with the insulin receptor, unlike other insulin sensitisers (15). The binding of glypican-4 to the insulin receptor regulates insulin activation and downstream signalling as an insulin sensitiser. Furthermore, circulating glypican-4 levels are higher in subjects with impaired glucose tolerance and positively correlated with BMI and WHR, the Homeostasis Model Assessment (HOMA) index (16).

Galanin and glypican-4 are likely to be main regulators in glucose and lipid metabolism and might be associated with insulin resistance and obesity, and PCOS is related with insulin resistance and other metabolic disorders such as dyslipidaemia, hypertension, endothelial dysfunction with reduced vascular compliance and, consequently atherosclerosis. In this context, it was analysed whether there was an alteration in serum galanin and glypican-4 levels in a group of women with and without PCOS. This study also set out to correlate both adipokine levels in relation with hormonal and metabolic profiles as well as cardiovascular risk factors to investigate the associations between some markers in connection with cardiovascular disease.

SUBJECTS AND METHODS

The design of the present study was approved by the Ethical Committee and Institutional Review Board of the Adnan Menderes University Faculty of Medicine, in accordance with the Declaration of Helsinki of the World Medical Association. After providing verbal information written informed consents were obtained from all women participating in the study.

This study applied G power analysis to calculate the sample size. Based on serum levels of galanin in the study by Bidzińska-Speichert and cols. (17) and on serum levels of glypican-4 in the study by Jędrzejuk and cols. (18), when the effect size for galanin (pg/mL) and glypican-4 (ng/mL) was 1.298 and 1.119, respectively; $\alpha = 0.05$ was the two-sided hypothesis, statistical power = 90%, a minimum of 17 subjects for galanin and 20 subjects for glypican-4 in each group should be studied. More patients were reached to increase the power of the study. A total of 44 women with PCOS and 44 age-matched controls were eligible. The Rotterdam criteria were applied for the diagnosis of PCOS (19) in the presence of at least two of the following: 1) oligomenorrhea and/or anovulation 2) biochemical and/or clinical hyperandrogenism 3) ultrasonographic appearance of polycystic ovaries (PCO) (multiple cysts > 12 in number of 2-9 mm size), along with the exclusion of other hormonal, metabolic and cardiovascular aetiologies (congenital adrenal hyperplasia, virilising ovarian or adrenal tumour, Cushing syndrome, hyperprolactinemia, diabetes mellitus, elevated blood pressure and any other cardiovascular diseases). No subject smoked or, consumed alcohol. Over 3 months preceding the study, none of the participants had been on hormonal contraceptives and any other medications or a diet which might affect lipid and carbohydrate metabolism. As controls, 44 age-matched healthy women, who had regular menses with no clinical and/or biochemical hyperandrogenism, were eligible.

A detailed clinical history was obtained, and physical examination was performed for all voluntary

women that participated in the present study. The BMI was calculated in kg/m^2 unit. Routine laboratory measurements were performed as follows: carbohydrate metabolism parameters including fasting blood glucose, fasting insulin, lipid profile including triglycerides (TG), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), pituitary hormones including follicle-stimulating hormone (FSH) and luteinising hormone (LH), sex steroids including oestradiol (E2), dehydroepiandrosterone sulphate (DHEA-SO4) and free testosterone (fT) and thyroid hormones including thyroid-stimulating hormone (TSH), free thyroxine levels (free T3 and free T4). Insulin resistance (IR) was determined by the Homeostasis Model Assessment (HOMA) index (fasting glucose (mg/dl) x fasting insulin (μU/mL)/405) (20). Phenotypical classification was performed as described previously (21). All sampling procedures were performed in the early follicular phase (day 2-5 of the menstrual cycle) in the morning after an overnight fast. In oligomenorrheic women samples were taken in their spontaneous cycle (day 2-5). Among the 44 patients with PCOS, only 2 had amenorrhea, in whom, progesterone withdrawal bleeding was achieved with medroxyprogesterone acetate 2*5 mg for 6 days (Tarlusal®, Deva Holding, Istanbul). Circulating hormone levels were measured in an auto-analyser (C8000 Architect, Abbott, Abbott Park, IL, USA) using the Chemiluminescent Microparticle Immunoassay (CMIA) method with Architect hormone kits. Serum galanin and glypican-4 levels were assessed by an enzyme-linked immunosorbent assay (Human GAL (Galanin) ELISA Kit, China). The intra- and inter-assay coefficients of variations for both adipokines were < 10%. The detection ranges for galanin and glypican-4 were 15.625-1000 pg/mL and 0.156-10 ng/mL, respectively.

Data analysis was performed using the software IBM SPSS Statistics version 17.0 (IBM Corporation, Armonk, NY, USA). Whether the distributions of continuous variables were normal or not was determined by the Kolmogorov Smirnov test. Data were expressed as mean ± SD or median (interquartile range), where applicable. The mean differences between control and PCOS groups were compared by Student's t test; otherwise, Mann Whitney's U test was applied for comparisons of the not normally distributed data. Degrees of association between continuous variables were evaluated by Spearman's Rank Correlation analyses. Whether the differences in galanin and glypican-4 levels between control and PCOS groups were keeping on or not was evaluated by Multiple Linear Regression Analyses after adjustment for all possible confounding factors. Any variable whose univariable test had a p value < 0.10 was accepted as a candidate for the multivariable model, along with all variables of known clinical importance. The coefficient of regression, 95% confidence interval and t-statistic for each independent variable were also calculated. Because they were not normally distributed, logarithmic transformation was used for both galanin and glypican-4 measurements in regression analyses. A p value less than 0.05 was considered statistically significant. However, for all possible multiple comparisons, Bonferroni Correction was applied for controlling Type I error. When Bonferroni adjustment was applied, a p value less than 0.025 was significant.

RESULTS

A total of 44 women with PCOS and 44 controls voluntarily participated. Among the 44 cases of PCOS, 29 (65.9%) women were phenotype A, 4 (9.1%) women were phenotype B, 5 (11.4%) women were phenotype C, and 6 (13.6%) women were phenotype D. The median galanin levels were lower (20.2 pg/mL vs. 26.4 pg/ mL, p = 0.002), whereas the median glypican-4 levels were higher (3.1 ng/mL vs. 2.6 ng/mL) in women with PCOS as compared to the women in the control group. Subgroup analyses regarding the BMI were also performed. Participants with BMI < 25 kg/m^2 were classified as lean, whereas women with a BMI ≥ 25 kg/m^2, comprised the overweight/obese group. Subgroup analysis indicated that the median galanin concentrations were lower in lean patients with PCOS (18 pg/mL vs. 23.3 pg/mL, p < 0.001) compared to controls. The PCOS patients with BMI ≥ 25 kg/m^2 also exhibited lower galanin concentrations, although this difference was not significant (38.4 pg/mL vs. 47.9 pg/mL, p = 0.043) after Bonferroni correction was applied. The galanin measurements of overweight and obese women were higher than those of lean women in both PCOS and control groups (38.4 pg/mL vs 18 pg/mL, p < 0.001 and 47.9 pg/mL and 23.3 pg/mL, p < 0.001, respectively). (Table 1, Figure 1). The glypican-4 levels were similar between PCOS and controls in the lean group (1.8 ng/mL vs. 1.2 ng/mL, p = 0.124), whereas overweight/obese PCOS women showed higher levels than the BMI- matched

controls (3.9 ng/mL vs. 2.9 ng/mL, p < 0.001). When PCOS and control groups were evaluated on their own, the glypican-4 levels were lower in lean women in both groups (1.8 ng/mL vs. 3.9 ng/mL, p < 0.001 and 1.2 ng/mL vs. 2.9 ng/mL, p < 0.001) (Table 1, Figure 2). The demographic, clinical, biochemical, hormonal and metabolic characteristics of women with PCOS and controls with regard to BMI are summarised in Table 1.

Circulating galanin concentrations were positively correlated with BMI, fasting insulin, triglyceride, glypican-4 levels and HOMA-IR and negatively correlated with LH levels when all participants were evaluated (p < 0.05). In the PCOS group, galanin showed a positive correlation with BMI, fasting insulin, triglyceride, free testosterone and glypican-4 levels, HOMA-IR and mFG scores; the correlation was negative with HDL levels. In controls, only BMI, mFG scores and glypican-4 levels were positively correlated with galanin levels (p < 0.025 according to the Bonferroni correction). Glypican-4 levels were positively correlated with BMI, fasting blood glucose, fasting insulin, triglyceride, free testosterone and galanin levels as well as HOMA-IR and mFG scores and negatively correlated with HDL and FSH levels in the total patient group (p < 0.05). In the PCOS group, glypican-4 levels were positively correlated with BMI, fasting insulin, triglyceride, free testosterone and, galanin levels, HOMA-IR and mFG scores. In controls, glypican-4 levels showed a positive correlation with galanin levels as well as age, BMI and mFG scores, whereas DHEA-SO4 and free testosterone levels were negatively correlated (p < 0.025 according to the Bonferroni correction). Tables 2 and 3 demonstrate the correlations of both galanin and glypican-4 levels with clinical, metabolic, hormonal and androgen excess parameters.

Table 1. Demographic, clinical, biochemical and hormonal characteristics of women with PCOS and controls regarding BMI

	PCOS			Controls							
	BMI<25 (n=24)	BMI≥25 (n=20)	Total (n=44)	BMI<25 (n=29)	BMI≥25 (n=15)	Total (n=44)	p^a	p^b	p^c	p^d	p^e
Age (years)	23.4±5.2	23.5±5.7	23.5±5.4	23.8±4.8	27.1±6.7	24.9±5.6	0.227†	0.786†	0.103†	0.936†	0.106†
BMI (kg/m²)			25.2±3.8			22.8±2.4	**<0.001**†				
FBG (mg/dL)	90.7±11	92.3±9.5	91.4±10.3	87.6±5.8	89.1±4.1	88.1±5.3	0.062†	0.220†	0.196†	0.615†	0.363†
FI (µIU/mL)	9.3(6.7)	12.8(9.9)	10.5 (8.8)	8(3.1)	10.1(6.5)	8.1(4)	**0.002**‡	0.174‡	**0.013**‡	**0.009**‡	0.173‡
HOMA-IR	2(1.4)	3(2.3)	2.4 (1.9)	1.7(0.8)	2.1(1.3)	1.8(0.9)	**<0.001**‡	0.136‡	**0.009**‡	**0.012**‡	0.110‡
TC (mg/dL)	183(46)	179(37.7)	180(45.2)	164(34.5)	186(39)	165.5(33.7)	0.108‡	0.088‡	0.882‡	0.944‡	0.162‡
HDL(mg/dL)	52.2±11.9	45.1±12.9	49±12.7	54.3±10.1	53.2±9.5	53.9±9.8	**0.045**†	0.498†	0.049†	0.064†	0.735†
LDL(mg/dL)	113.6±20.8	112.8±29.6	113.3±24.9	97±19	109.3±25.6	101.2±22	**0.018**†	**0.004**†	0.710†	0.920†	0.079†
TG (mg/dL)	92.5(59.5)	119(104.5)	95.5(62)	77(31)	93(45)	80.5(41.2)	0.027‡	0.153‡	0.149‡	0.075‡	0.116‡
fT(pg/mL)	2.6(1)	4(1.1)	3(1.9)	1.6(1.2)	1.1(0.8)	1.2(1.1)	**<0.001**‡	**<0.001**‡	**<0.001**‡	**<0.001**‡	**0.023**‡
DHEA-SO4 (µg/dL)	300.5(220.7)	411(268.5)	330(244.2)	272(127.5)	241(177)	258.5(128.2)	**0.002**‡	0.155‡	**0.002**‡	0.487‡	0.081‡
fT3 (pg/mL)	3.2±0.4	3.1±0.6	3.1±0.5	3.1±0.5	2.9±0.3	3±0.4	0.221†	0.347†	0.358†	0.584†	0.418†
fT4 (pg/mL)	1.1(0.3)	1.1(0.2)	1.1(0.2)	1.1(0.2)	1.1(0.2)	1.1(0.2)	0.350‡	0.537‡	0.419‡	0.654‡	0.496‡
TSH (µIU/mL)	1.6(1.5)	1.7(1.5)	1.7(1.5)	1.8(1.1)	1.6(0.8)	1.7(1)	0.917‡	0.830‡	0.633‡	0.715‡	0.683‡
FSH (mIU/mL)	4.2(1.2)	3.9(1.6)	4.2(1.2)	4.7(1.6)	4.5(1.2)	4.7(1.4)	**0.017**‡	0.062‡	0.191‡	0.311‡	0.552‡
LH (mIU/mL)	7.6(9.4)	7(7.2)	7.2(8)	4.8(2.5)	5(1.5)	4.8(1.9)	**<0.001**‡	**<0.001**‡	**0.011**‡	0.494‡	0.990‡
E2 (pg/mL)	47.5(43.5)	46(39.8)	47.5(38)	65(32.5)	70(48)	65(37.2)	**0.047**‡	0.198‡	0.107‡	0.680‡	0.594‡
PRL (mIU/mL)	15±3	15.9±5.2	15.4±4.1	16.1±4.4	15.1±4.1	15.7±4.3	0.727†	0.306†	0.617†	0.484†	0.492†
mFG	9(6.7)	11(3)	10(5.2)	3(2)	4(1)	3(1)	**0.047**‡	**<0.001**‡	**<0.001**‡	**<0.001**‡	**<0.001**‡
Galanin (pg/mL)	18(2)	38.4(19.9)	20.2(18.8)	23.3(4.3)	47.9(17)	26.4(16.2)	**0.002**‡	**<0.001**‡	0.043‡	**<0.001**‡	**<0.001**‡
Glypican-4 (ng/mL)	1.8(1.4)	3.9(1)	3.1(2.1)	1.2(2.3)	2.9(0.2)	2.6(2.2)	**<0.001**‡	0.124‡	**<0.001**‡	**<0.001**‡	**<0.001**‡

BMI: body mass index; FBG: fasting blood glucose; FI: fasting insulin; HOMA-IR: Homeostasis Model Assessment-Insulin resistance; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides; fT: free testosterone; DHEA-SO4: dehydroepiandrosterone sulphate; TSH: thyroid stimulating hormone; FSH: follicle stimulating hormone; LH: luteinizing hormone; E2: estradiol; PRL: prolactin; mFG: modified Ferriman–Gallwey.

p^a difference between PCOS and controls. p^b difference between PCOS and controls BM I <25, p value less than 0.025 was considered statistically significant with regard to Bonferroni adjustment. p^c difference between PCOS and controls BMI ≥25, p value less than 0.025 was considered statistically significant with regard to Bonferroni adjustment. p^d difference between BMI <25 and BMI ≥25 subgroups in patients with PCOS, p value less than 0.025 was considered statistically significant with regard to Bonferroni adjustment. p^e difference between BMI <25 and BMI ≥25 subgroups in controls, p value less than 0.025 was considered statistically significant with regard to Bonferroni adjustment. † Student's *t* test. ‡ Mann Whitney U test.

The best predictor(s) which effect both galanin and glypican-4 concentrations were also evaluated by Multiple Linear Regression analyses, after adjustment for all possible confounding factors. As a result of univariate statistical analysis, variables with $p < 0.01$ were included in the linear regression model as candidate risk factors. Since galanin and glypican-4 levels were distributed far from normal, logarithmic

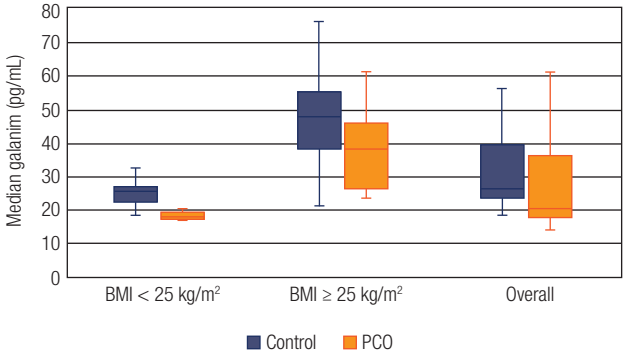

Figure 1. Galanin levels in PCOS and controls regarding BMI. The horizontal lines in the middle of each box indicates the median galanin levels, while the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark indicates the maximum and minimum levels, respectively.

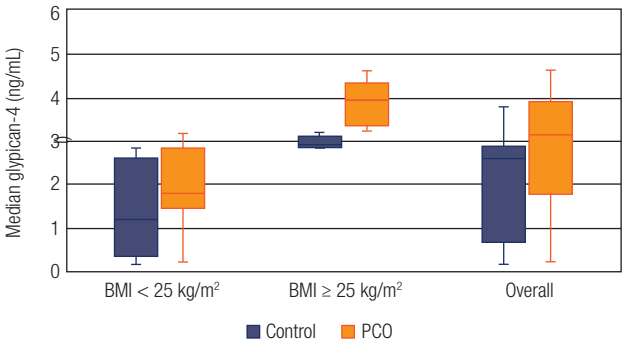

Figure 2. Glypican-4 levels in PCOS and controls regarding BMI. The horizontal lines in the middle of each box indicates the median glypican-4 levels, while the top and bottom borders of the box mark the 25th and 75th percentiles, respectively. The whiskers above and below the box mark indicates the maximum and minimum levels, respectively.

Table 2. Correlations between galanin levels and clinical, metabolic, hormonal and androgen excess parameters

	All patients (n=88)		PCOS (n=44)		Controls (n=44)	
	r	p[a†]	r	p[b†]	r	p[b†]
Age (years)	0.087	0.422	-0.028	0.858	0.149	0.335
BMI (kg/m²)	0.689	**<0.001**	0.966	**<0.001**	0.768	**<0.001**
FPG (mg/dL)	0.017	0.872	0.100	0.519	0.108	0.484
FI (µIU/mL)	0.216	**0.043**	0.488	**<0.001**	0.199	0.196
HOMA-IR	0.211	**0.048**	0.474	**<0.001**	0.217	0.157
TC (mg/dL)	0.025	0.819	-0.094	0.546	0.252	0.099
HDL(mg/dL)	-0.192	0.072	-0.467	**<0.001**	-0.009	0.953
LDL(mg/dL)	-0.063	0.557	-0.114	0.463	0.187	0.224
TG(mg/dL)	0.230	**0.031**	0.358	**<0.001**	0.284	0.062
fT(pg/mL)	-0.007	0.946	0.652	**<0.001**	-0.124	0.423
DHEA-SO4 (µg/dL)	-0.069	0.520	0.170	0.269	-0.076	0.622
fT3 (pg/mL)	-0.102	0.344	0.052	0.738	-0.174	0.260
fT4 (pg/mL)	-0.155	0.151	-0.032	0.836	-0.175	0.255
TSH (µIU/mL)	-0.017	0.873	0.104	0.503	-0.204	0.185
FSH (µIU/mL)	-0.111	0.302	-0.149	0.336	-0.325	0.031
LH (µIU/mL)	-0.222	**0.038**	-0.044	0.776	-0.085	0.584
E2 (pg/mL)	0.089	0.410	-0.101	0.513	0.162	0.295
PRL (µIU/mL)	0.122	0.259	0.200	0.192	0.017	0.913
mFG	0.202	0.059	0.693	**<0.001**	0.407	**0.006**
Glypican4 (ng/mL)	0.651	**<0.001**	0.965	**<0.001**	0.770	**<0.001**

BMI: body mass index; FPG: fasting plasma glucose; FI: fasting insulin; HOMA-IR: Homeostasis Model Assessment-Insulin resistance; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides; fT: free testosterone; DHEA-SO4: dehydroepiandrosterone sulphate; fT3-fT4: free thyroxine levels; TSH: thyroid stimulating hormone; FSH: follicle stimulating hormone; LH: luteinizing hormone; E2:estradiol; PRL: prolactin; mFG: modified Ferriman–Gallwey.

[a]p value less than 0.05 was considered statistically significant; [b]p value less than 0.025 was considered statistically significant with regard to Bonferroni adjustment. r: Correlation coefficient; †Spearman's Rank Correlation test.

Table 3. Correlations between glypican-4 levels and clinical, metabolic, hormonal and androgen excess parameters

	All patients (n=88)		PCOS (n=44)		Controls (n=44)	
	r	p[a†]	r	p[b†]	r	p[b†]
Age (years)	0.175	0.104	0.057	0.713	0.434	**0.003**
BMI (kg/m^2)	0.971	**<0.001**	0.999	**<0.001**	0.999	**<0.001**
FPG (mg/dL)	0.230	**0.031**	0.155	0.314	0.242	0.114
FI (µIU/mL)	0.433	**<0.001**	0.463	**0.002**	0.231	0.131
HOMA-IR	0.457	**<0.001**	0.455	**0.002**	0.281	0.064
TC (mg/dL)	0.095	0.377	-0.110	0.477	0.302	0.046
HDL (mg/dL)	-0.334	**<0.001**	-0.508	**<0.001**	0.086	0.581
LDL (mg/dL)	0.099	0.358	-0.120	0.436	0.270	0.077
TG (mg/dL)	0.292	**0.006**	0.366	**0.015**	0.111	0.475
fT (pg/mL)	0.348	**<0.001**	0.631	**<0.001**	-0.339	**0.024**
DHEA-SO4	0.036	0.736	0.133	0.388	-0.363	**0.015**
fT3 (pg/mL)	0.027	0.800	0.101	0.515	-0.263	0.085
fT4 (pg/mL)	-0.065	0.546	-0.065	0.673	-0.154	0.317
TSH (µIU/mL)	0.012	0.911	0.121	0.433	-0.126	0.414
FSH (µIU/mL)	-0.228	**0.033**	-0.178	0.247	-0.132	0.393
LH (µIU/mL)	0.085	0.433	-0.049	0.753	-0.140	0.365
E2 (pg/mL)	-0.164	0.127	-0.157	0.310	0.067	0.664
PRL (µIU/mL)	0.055	0.613	0.147	0.340	-0.088	0.569
mFG	0.645	**<0.001**	0.668	**<0.001**	0.526	**<0.001**
Galanin (pg/mL)	0.651	**<0.001**	0.965	**<0.001**	0.770	**<0.001**

BMI: body mass index; FPG: fasting plasma glucose; FI: fasting insulin; HOMA-IR: Homeostasis Model Assessment-Insulin resistance; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; TG: triglycerides; fT: free testosterone; DHEA-SO4: dehydroepiandrosterone sulphate; fT3-fT4: free thyroxine levels; TSH: thyroid stimulating hormone; FSH: follicle stimulating hormone; LH: luteinizing hormone; E2: estradiol; PRL: prolactin; mFG: modified Ferriman–Gallwey.

[a]p value less than 0.05 was considered statistically significant; [b]p value less than 0.025 was considered statistically significant with regard to Bonferroni adjustment. r: Correlation coefficient; †Spearman's Rank Correlation test.

transformation for both measurements was performed in the regression analysis. As there was a functional connection between fasting blood glucose and insulin levels and HOMA-IR, only HOMA-IR was included in the regression model instead of fasting blood glucose and insulin. Multiple Linear Regression analyses showed that PCOS and BMI appear to be independent risk factors affecting galanin levels ($p < 0.001$). When the correction was made according to other factors, the effect of being in the PCOS group compared to the control group continued to decrease the level of galanin ($B = -0.472$, 95%CI: -0.577 to -0.368 and $p < 0.001$). In addition, as the BMI increased, galanin levels continued to increase independently of other factors ($B = 0.113$, 95%CI: 0.097-0.129 and $p < 0.001$). Regarding the glypican-4 levels, the BMI was an independent risk factor ($p < 0.001$), and the significant effect of PCOS on glypican-4 levels in the univariate analysis disappeared in Multiple Linear Regression analyses ($p = 0.181$). As the BMI increased, the glypican-4 levels continued to increase independently of other factors ($B = 0.220$, 95%CI: 0.162-0.277 and $p < 0.001$); Table 4).

DISCUSSION

Our results show that patients with PCOS exhibited lower galanin and higher glypican-4 levels than controls. However, when the subjects were further divided based on the BMI, subgroup analyses showed that galanin levels were significantly lower in lean patients with PCOS as compared to the BMI-matched controls, whereas this difference did not reach significance in the overweight/obese group. As for glypican-4 levels, the overweight/obese PCOS group demonstrated higher levels than the controls, whereas lean groups yielded similar results. Both adipokine levels showed positive correlations with BMI as well as metabolic syndrome markers and androgenic profile; however, multivariate analyses demonstrated that PCOS and BMI appear to

Table 4. Multiple Regression Analysis of Possible Factors Affecting Galanin and Glypican-4 Levels

	Coefficient of regression	95% Confidence Interval		t-statistic	p value
		Lower limit	Upper limit		
Galanin					
PCO	-0.472	-0.577	-0.368	-8.998	**<0.001**
BMI	0.113	0.097	0.129	14.170	**<0.001**
HOMA-IR	-0.007	-0.022	0.009	-0.863	0.391
HDL cholesterol	0.001	-0.002	0.005	0.714	0.477
Triglyceride	0.0005	-0.0004	0.001	1.106	0.272
LH	0.005	-0.001	0.011	1.650	0.103
mFG	-0.005	-0.023	0.012	-0.626	0.533
Glypican					
PCO	0.270	-0.128	0.667	1.350	0.181
BMI	0.220	0.162	0.277	7.565	**<0.001**
HOMA-IR	-0.016	-0.072	0.040	-0.556	0.580
HDL cholesterol	0.005	-0.008	0.017	0.722	0.472
Triglyceride	-0.001	-0.004	0.002	-0.895	0.373
fT	-0.134	-0.315	0.047	-1.475	0.144
FSH	0.029	-0.061	0.119	0.638	0.525
mFG	0.004	-0.062	0.070	0.122	0.903

be independent risk factors affecting galanin levels, while only BMI was an independent risk factor affecting glypican-4 levels.

Galanin is involved in appetite, obesity, dyslipidaemia, insulin resistance and diabetes mellitus, hypertension, metabolic syndrome as well as reproduction (5,10). Numerous studies pointed out the relationship between galanin and metabolic syndrome. As a result, it can be used as a cardiovascular disease marker, demonstrating higher levels of this adipokine in diabetes mellitus, impaired glucose tolerance and gestational diabetes mellitus (22-24). Acar and cols. (25) reported that galanin levels were positively correlated with insulin resistance and triglycerides, also in obese children. Interestingly, physical activity is an effective stimulus to enhance galanin secretion (26). Fang and cols. (27) concluded that the galanin system is required for physical activity to relieve insulin resistance, causing a beneficial effect on exercise-induced GLUT-4 translocation. Galanin resistance, which is defined to be the discrepancy between high levels of circulating galanin and low glucose handling in the diabetic population, is the critical step in the development of type 2 diabetes mellitus (28). In contrast, galanin can increase insulin sensitivity, but the circulating levels of galanin are high in the diabetic group. Galanin resistance is thought to be highly related to obesity. However, there are very few studies about galanin levels in patients with PCOS. Baranowska and cols. (29) reported that galanin concentrations in PCOS were higher than those in the control group, but the difference was not significant statistically. Similarly, Bidzińska-Speichert and cols. (17) found that patients with PCOS, both obese (BMI ≥ 30) and non-obese (BMI < 30) had lower levels of galanin, similar to our data. The high galanin levels in the diabetic group suggest galanin resistance. However, the circulating levels were lower in PCOS patients, contrary to what was expected. Our results lead us to infer that there is a linkage between insulin resistance in PCOS disease and galanin deficiency. As galanin is an important hormone for elevating insulin sensitivity via GLUT-4 translocation (5,27), perhaps galanin deficiency may at least be one of the efficient factors responsible for insulin resistance in PCOS. Since galanin elevates insulin sensitivity via causing an increase in GLUT-4 translocation and a decrease in insulin secretion from the pancreas (5), the results of the present study made us hypothesise that galanin deficiency is associated with insulin resistance in the group of PCOS patients. Galanin resistance might not be valid in the PCOS group, unlike the diabetic group, presumably because of the young age of these women. However, as they

get older and start to develop type 2 diabetes, galanin resistance arise, resulting in higher levels. Another reason might be that galanin resistance is also highly related to obesity; very few patients in the present study were obese, with a mean BMI of 25.2 kg/m^2. Galanin is a target agent for sex steroids, serving as a molecular motifs integrating the control of metabolism as well as reproduction (9), and leading to an alteration in gonadotropin-releasing hormone (GnRH) secretion (30). It stimulates LH secretion in porcine, but inhibits it in the mice (11,12). Possibly, it interacts with LH in humans, such as in mice, so that LH levels could not be inhibited because of lower galanin levels in women with PCOS. Our results show that galanin levels are negatively correlated with LH levels.

Glypican-4 is also a newly identified adipokine, a cell surface proteoglycan, which interacts directly with the insulin receptor (15). Binding of glypican-4 to the insulin receptor regulates insulin activation and downstream signalling as an insulin sensitiser. *In-vitro* studies have found that when glypican-4 depletes, insulin receptor activation diminishes (15). Circulating levels are correlated with BMI and associated with insulin sensitivity (15). Recently, Ning and cols. (31) stated, for the first time, that serum glypican-4 levels were elevated in subjects with metabolic syndrome. Their data also showed a positive correlation with fasting blood glucose, fasting insulin and HOMA-IR in all subjects, in compliance with the data of the current study. Circulating glypican-4 levels are higher in subjects with impaired glucose tolerance and positively correlated with BMI, WHR and HOMA index (16). Leelalertlauw and cols. (32) also found elevated glypican-4 levels in obese children, increasing with higher degrees of obesity. Yoo and cols. (33) mentioned a gender-based difference in glypican-4 levels, demonstrating higher levels in men than in women. They declared a positive relationship between glypican-4 levels and WHR as well as the ratio of visceral to subcutaneous fat area. These authors also stated that glypican-4 levels correlated with cardiometabolic risk factors, including insulin resistance and arterial stiffness, and the measurements were independently associated with non-alcoholic fatty liver disease, in women. Nevertheless, there is only one pilot study evaluating glypican-4 levels in women with PCOS. Jędrzejuk and cols. (18) conducted a pilot study to determine the connection between glypican-4 and cardiovascular parameters in patients with PCOS. They concluded that glypican-4 levels were higher in women with PCOS than those of controls and correlated with cardiovascular risk factors, in particular fat distribution, in spite of the low mean BMI of 22 kg/m^2 and the absence of lipid disorders in the evaluated subjects. Their results revealed a positive correlation with metabolic parameters, including insulin and HOMA-IR, as well as androgenic markers, similar to the present study. In our study, Multiple Linear Regression analyses showed that BMI was an independent risk factor for glypican-4 levels, and the significant effect of PCOS on glypican-4 levels in the univariate analysis disappeared. Little is known about the signalling functions of glypican-4; the role of glypican-4 in adipocytes and its relationship to metabolic regulation remains unknown. Since the intracellular post-receptor events after binding to the receptor are not exactly known, we think that further molecular studies should be done on this subject. Based on these data, it is assumed that, when insulin resistance is present in a subject, glypican-4 levels may increase to enhance insulin sensitivity, if glypican-4 is acting as an insulin sensitivity-enhancing agent. This way, glypican-4 agonists may be used as treating agents. Consequently, our data reveal that serum galanin levels are lower and glypican-4 levels are higher in women with PCOS as compared to controls. Further studies are needed to determine whether these adipokines could be used as additional markers for insulin sensitivity and lipid profile and whether they might play a role in the pathogenesis of PCOS, in which the risks of metabolic cardiovascular risks are increased. Multivariate analyses suggest that PCOS and BMI may be the best predictors affecting galanin levels with a decreasing and increasing effect, respectively; in turn, BMI was the best predictor affecting glypican-4 levels with an increasing effect. Our results suggest the use of innovative medical treatment options of insulin resistance in further studies. As, galanin deficiency might at least be one of the efficient factors associated with insulin resistance in PCOS, it is hypothesised that the administration of galanin can increase insulin sensitivity. Further research may also use glypican-4 agonists to relieve insulin resistance and androgen excess. Regarding the prospective design, a small sample size may result in limitations. In this study, it was not possible to subdivide the phenotypic characteristics in the patient group due to the small sample. Nevertheless, altered levels of these adipokines in relation with PCOS in even young and lean women suggest that the mechanisms related to these pathways require further studies.

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Adrenal crisis and mortality rate in adrenal insufficiency and congenital adrenal hyperplasia

[1] Unidade de Endocrinologia do Desenvolvimento, Laboratório de Hormônios e Genética Molecular (LIM42), Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil

Lia Mesquita Lousada[1]
https://orcid.org/0000-0002-1170-253X

Berenice B. Mendonca[1]
https://orcid.org/0000-0003-1762-1084

Tania A. S. S. Bachega[1]
https://orcid.org/0000-0002-5219-3153

ABSTRACT

Primary adrenal insufficiency (PAI) is characterized by the inability of the adrenal cortex to produce sufficient amounts of glucocorticoids and/or mineralocorticoids. Addison's disease (AD) and congenital adrenal hyperplasia (CAH) are the most frequent disorders in adults and children, respectively. Despite the diagnostic advances and the availability of glucocorticoid and mineralocorticoid replacements, adrenal crisis (AC) is still a potentially lethal condition contributing to the increased mortality, not only during the first year of life, but also throughout life. Failure in increasing glucocorticoid doses during acute stress, when greater amounts of glucocorticoids are required, can lead to AC and an increase morbimortality rate of PAI. Considering a mortality rate of 0.5 per 100 patient years, up to 1,500 deaths from AC are expected in Brazil in the coming decade, which represents an alarming situation. The major clinical features are hypotension and volume depletion. Nonspecific symptoms such as fatigue, lack of energy, anorexia, nausea, vomiting, and abdominal pain are common. The main precipitating factors are gastrointestinal diseases, other infectious disease, stressful events (e.g., major pain, surgery, strenuous physical activity, heat, and pregnancy), and withdrawal of glucocorticoid therapy. Suspected AC requires immediate therapeutic action with intravenous (iv) hydrocortisone, fluid infusion, monitoring support, and antibiotics if necessary. AC is best prevented through patient education, precocious identification and by adjusting the glucocorticoid dosage in stressor situations. The emergency card, warning about acute glucocorticoid replacement, has high value in reducing the morbidity and mortality of AC. Arch Endocrinol Metab. 2021;65(4):488-94

Keywords

Primary adrenal insufficiency; congenital adrenal hyperplasia; mortality; adrenal crisis; emergency care

Correspondence to:
Lia Mesquita Lousada
Laboratório de Hormônios e Genética Molecular (LIM/42)
Av. Dr. Enéas de Carvalho Aguiar, 155, 2º andar, bloco 6, Cerqueira César
05403-900 – São Paulo, SP, Brasil

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INTRODUCTION

Primary adrenal insufficiency (PAI), first described by Thomas Addison in 1855, is characterized by the inability of the adrenal cortex to produce enough glucocorticoids and/or mineralocorticoids. The prevalence of PAI is around 82-144/million and the most common causes are autoimmunity (Addison's disease [AD]) in adults and genetic causes in children, especially enzymatic defects (congenital adrenal hyperplasia [CAH]) (1). These disorders are potentially life-threatening conditions due to the central role of glucocorticoids and/or mineralocorticoids in energy, salt, and fluid homeostasis (2).

Prior to Addison's time, adrenal insufficiency was an invariably fatal condition due to absence of steroid replacement therapy, with a 1-year survival rate of about 20% or less. Most patients died within the first 5 years after diagnosis. The discovery of cortisone by Hench, Kendall, and Reichstein in the late 1940s improved the survival rate dramatically. Initial data demonstrated that the life expectancy of adrenal insufficiency patients was similar to that of the general population, except when the disease was undiagnosed and patients were under poor social conditions (3,4).

Nevertheless, further studies among hypopituitarism patients demonstrated excessively high mortality, possibly due to inadequate glucocorticoid replacement therapy. These data stimulated additional studies about mortality rate due to adrenal insufficiency (5). Most available data came from retrospective studies. A Swedish study (n = 1,675 patients) demonstrated increased mortality in AD patients: a mortality rate 2-fold higher than that of the reference population (RR: 2.19 in males and 2.86 in females) (6). Although

a Norwegian series (n = 811 patients) suggested no significant difference in mortality rate for the whole group, those patients diagnosed before 40 years old presented an increased mortality rate at 1.50 (95% CI 1.09-2.01), especially among males (2.03 [1.19-2.86]). Adrenal insufficiency was the major cause of death (15% of 130 deceased patients), most likely during adrenal crisis (AC). The mean age of death was 75.7 years for females and 64.8 years for males, representing, respectively, 3.2 and 11.2 years less than the estimated life expectancy (7).

Among children, the salt-wasting (SW) form of CAH due to 21-hydroxylase deficiency commonly presents as a neonatal SW crisis characterized by hyponatremic dehydration and, if untreated, death. Although the simple virilizing (SV) form of CAH does not usually present as a spontaneous SW crisis, special attention should be given during stressful events (8).

Prior to the introduction of newborn screening (NBS) programs for CAH, the neonatal mortality was higher, especially among SW males. This increased mortality was suggested by both the low proportion of the SW form in relation to the SV form and the low male-to-female ratio in the Hospital das Clínicas cohort, as well as in other unscreened populations (9-11). The CAH NBS program has been applied in Sao Paulo/Brazil since 2013. The effectiveness of this public program was demonstrated by the increasing number of male patients, reaching a similar proportion of affected males and females, as expected for an autosomal recessive disorder, and by the increasing frequency of SW in relation to SV patients (70% to 90%) (12-14).

Despite the earlier CAH diagnosis, through clinical means or NBS, the mortality rate remained high. Three retrospective studies comprising 1,191 English and Swedish CAH patients evidenced increased mortality in all age groups, varying from 2- to 5-fold in comparison with the general population (15-17).

In all these studies on mortality in adrenal insufficiency, the main causes of death were cardiovascular disease, AC, infections, and cancer. Inappropriate glucocorticoid replacement therapy, whether over- or underdosing treatment, corresponds with increased mortality in adrenal insufficiency patients. Lifelong overdosing glucocorticoid replacement therapy could be related to adverse effects such as increased frequency of cardiovascular disease, metabolic syndrome, infections, or cancer. However, insufficient glucocorticoid replacement during stress events and simultaneous illnesses can induce AC (6,7,18).

Notwithstanding the adrenal insufficiency diagnostic advances and the availability of glucocorticoid and mineralocorticoid replacements, these studies illustrate that AC is still a potentially lethal condition that contributes to the high mortality rate in adrenal insufficiency, not only during the first year of life, but also throughout life (15). In the Hospital das Clínicas cohort, 4 out of 250 classical CAH patients and 2 adult patients with bilateral adrenalectomy died due to inadequate glucocorticoid replacement during illness (unpublished data). Although there are few case reports of death due to undiagnosed adrenal insufficiency and AC in the literature (19-21), we hypothesize that the number of deaths from undiagnosed adrenal insufficiency and AC is higher than previously noticed due to difficulty in collecting real-world data (22). Since adrenal insufficiency and AC aren't so frequent, health professionals may not be familiar with the precocious diagnoses and treatment. These data highlight the necessity of continuous education of patients, relatives, caregivers, and physicians, emphasizing the importance of stress hydrocortisone doses during adverse events (23).

ADRENAL CRISIS (AC)

Pathophysiology of AC

It is well known that during stressful events, such as fever and infection, endogenous cortisol levels increase substantially in subjects with preserved adrenal function. Infection triggers the release of cytokines such as interleukin 1 (IL-1), tumor necrosis factor α (TNFα), and interleukin 6 (IL-6), which stimulate the hypothalamus-pituitary-adrenal (HPA) axis to increase the cortisol levels. As a feedback mechanism, the high glucocorticoid levels decrease the production of inflammatory cytokines to avoid exacerbated deleterious effects (24).

Patients with PAI and CAH are at risk of life-threatening AC due to their inability to intensify glucocorticoid production during stress (2,25,26). AC is more frequent in patients with PAI than in those with secondary adrenal insufficiency, possibly due to partial preservation of cortisol secretion in some patients with secondary adrenal insufficiency, as well as mineralocorticoid deficiency in those with PAI (26).

Clinical manifestations and diagnosis of AC

Allolio and cols. defined AC, also called acute adrenal insufficiency or Addisonian crisis, as a profound impairment of general health and at least two of the following conditions: hypotension (systolic blood pressure < 100 mmHg), nausea or vomiting, severe fatigue, hyponatremia, hypoglycemia, hyperkalemia, and reversibility after administration of parenteral glucocorticoids (27).

The major clinical features of AC are volume depletion and hypotension. Volume depletion is caused by a failure to retain fluid and sodium due to the deficiency of mineralocorticoid action, which is prominent in PAI but not in secondary adrenal insufficiency. The volume depletion can be intensified by vomiting and diarrhea (26,28). Glucocorticoids exert a permissive effect for catecholamine action in the endothelium vessels and cardiac tissue during stress activation of the cardiovascular system. Therefore hypotension occurs secondarily to both hypovolemia and hypocortisolism (29). If hypotension first attributed to AC does not reverse after glucocorticoid parenteral infusion, the coexistence of other conditions associated with hypotension, such as sepsis, must be considered (26).

Nonspecific symptoms such as fatigue and lack of energy are common. Anorexia, nausea, vomiting, and abdominal pain are also observed in AC, being frequently misdiagnosed as gastrointestinal disease (25,27). Fever is frequently current, as many AC episodes are triggered by infection and due to increased inflammatory cytokines (30). At a later stage, impaired cognition and somnolence may occur (28). Hypoglycemia, more frequent in children and rare in adults, may occur due to reduced gluconeogenesis related to hypocortisolism. Cortisol deficiency may also lead to neutropenia, eosinophilia, and lymphocytosis. Combined glucocorticoid and mineralocorticoid deficiencies can result in urinary sodium loss, hyponatremia, hyperkalemia, and increased serum urea (2,26,31). Other long-term manifestations include hyperpigmentation related to hypersecretion of proopiomelanocortin-derived peptides (only in PAI), orthostatic hypotension, and, in children, failure to thrive (2).

The diagnosis of adrenal insufficiency is often delayed due to the insidious onset and nonspecific symptoms (weakness, fatigue, musculoskeletal pain, weight loss, abdominal pain, depression, and anxiety). Some patients are misdiagnosed as having psychiatric illness (e.g., nervous anorexia) (28) or gastrointestinal disease (1,27).

Incidence and mortality of AC

Although patient-initiated stress dosing seems to be effective to avert AC, the incidence of these events remains elevated. Two large retrospective postal survey studies comprising 1,287 adult patients with adrenal insufficiency from Germany, the United Kingdom, Canada, Australia, and New Zealand evidenced an AC incidence of around 6.3-8/100 patient years (25,32).

Discouraging results were seen even among patients instructed about prevention of AC. A 2-year German prospective study (n = 423 patients) analyzed the occurrence of AC in patients with adrenal insufficiency who had received detailed written instructions on glucocorticoid stress dose adjustments. The AC incidence was 8.3/100 patient years. Approximately one in 12 patients with adrenal insufficiency will experience a life-threatening crisis in the coming year, and patients with a previous AC are at 2.8-fold risk to develop later episodes. The main precipitating factors were gastrointestinal diseases, other infectious diseases, emotional stress, and stressful events (e.g., major pain, surgery, strenuous physical activity, heat, and pregnancy). In 7% of AC episodes, a precipitating factor was not identified. Four out of 10 deaths were associated with AC, 0.5 AC-related deaths per 100 patient years, and the AC mortality was approximately 6% (33).

Rushworth and cols. also cited older age, a prior AC, the presence of autoimmune polyglandular syndromes, type 1 diabetes mellitus, and nonendocrine coexisting conditions such as asthma and cardiac disease as AC risk factors among patients with hypoadrenalism (26). Inadequate discontinuance of glucocorticoid therapy by the patient (or by an attending physician) is also a triggering factor of AC (27).

Failure of adequate preventive measures and prompt diagnosis of AC by patients and physicians is common. A long-term Australian study analyzed all attendances between 2000 and 2017 of 56 PAI adult patients in a large regional referral center (252 attendances). Nearly half (45.2%, 114 out of 252) of the attendances were related to an infection. Only 2.8% (7 out of 252) used intramuscular (IM) hydrocortisone

prior to presentation, and just 17.9% (45 out of 252) of the hospital presentations followed any form of stress dosing. The treating clinicians diagnosed 61 AC episodes (24.4%, 61out of 252). Among patients with a clinician-diagnosed AC, only 32.8% (20 out of 61) had used stress dosing before presentation (34).

Assuming an adrenal insufficiency prevalence of 82-144/million (1) and estimating the Brazilian population in 2020 at 211,755,692 people (https://cidades.ibge.gov.br/brasil/panorama),

17,363 to 30,492 Brazilians may be affected by adrenal insufficiency. As mortality from AC is estimated at 0.5/100 patient years (33), 868 to 1,524 deaths from AC are expected in Brazil in the coming decade. These data represent an alarming context regarding morbimortality and costs for public health systems, mainly because adrenal crisis is a preventable condition.

Among children, the incidence and mortality of AC is similar to that in adult patients: 5-10 episodes/100 patient years, with 1 death in every 200 episodes of AC (35).

Among CAH patients, AC is common even after the neonatal period. In a cross-sectional questionnaire-based study of 122 CAH patients, the AC frequency was 5.8/100 patient years in the whole group and 4.9/100 patient years after correction for neonatal SW crisis, with no difference in incidence between males and females. AC episodes mostly occurred during childhood, with one-third occurring in the first year and more than 70% within the first 10 years of life. Still, 20% of adrenal events were observed in adults. An age-related pattern was observed, with respiratory infections being the main trigger in early childhood, whereas gastrointestinal infections were the main cause at older ages. In this study, the median time for recognition of the first AC symptoms was one day, even in chronic, well-informed patients (36).

Management and treatment of AC

Suspected AC requires immediate therapeutic action (27). In acute sick patients with clinical signs suspected of AC, treatment should not be delayed awaiting test results. Before hydrocortisone administration, a single basal adrenocorticotropic hormone (ACTH) and cortisol sample collection, at any time of the day, is essential for the diagnosis (2).

For adults, immediate parenteral injection of hydrocortisone (100 mg), as well as a fluid restoration with 1,000 mL of isotonic saline within the first hour, should be performed in cases of suspected AC. Subsequently, 200 mg of hydrocortisone should be administered during the first 24 hours (6 hourly injections or continuous iv administration). During the following day, the hydrocortisone dose should be reduced to 100 mg and, afterward, switched to a double oral glucocorticoid regimen depending on the clinical state. Prednisolone (25 mg as a bolus, followed by two 25 mg doses, for a total of 75 mg in the first 24 hours; thereafter, 50 mg every 24 hours) is an alternative if hydrocortisone is unavailable; dexamethasone should be avoided, only being given (4 mg every 24 hours) if no other glucocorticoid is available (Table 1) (2,26,27,37-39).

Table 1. Management of adrenal crisis

Topic	Treatment
1	Volume expansion: 1,000 mL iv isotonic saline within the first hour. Children: 20 mL/kg isotonic saline. Can repeat up to a total of 60 mL/kg within 1 h for shock.
2	100 mg iv hydrocortisone followed by 200 mg/d hydrocortisone as a continuous infusion for 24 h or every 6 h, reduced to 100 mg/d hydrocortisone the following day. Children: 50-100 mg/m^2 iv hydrocortisone bolus followed by 50-75-100 mg/m^2/d hydrocortisone every 6 h or 2-3 mg/m^2/h as a continuous infusion for 24 h. *Alternative simpler scheme of bolus stress doses of hydrocortisone: children < 15 kg (0-2 years), 25 mg; 15-25 kg (2-6 years), 50 mg; and > 25 kg (>6 years), 100 mg.
3	For hypoglycemia: 0.5-1 g/kg dextrose or 2-4 mL/kg of 25% dextrose solution (maximum single dose 25 g) infused slowly at a rate of 2 to 3 mL/min. Alternatively, 5-10 mL/kg of 10% dextrose solution for children < 12 years old
4	Cardiac and hemodynamic monitoring. Low heparin doses. Antibiotics if necessary.
5	Switch to oral regimen depending on clinical state.

Adapted from: Bornstein et al. (2), Allolio (27), Miller et al. (35), Nowotny et al. (39) and Shulman et al. (40)*.

For children, 50-100 mg/m^2 of hydrocortisone as an initial stress dose and fluid restoration with 20 mL/kg of isotonic saline within the first hour should administered in cases of suspected AC (35). The European Reference Network on Rare Endocrine Conditions (EndoERN) suggests an alternative scheme of hydrocortisone bolus stress doses based on the patient's age and weight: children < 15 kg (0-2 years), 25 mg; 15-25 kg (2-6 years), 50 mg; and > 25 kg (>6 years), 100 mg, as in adults (39). Subsequently, 50-75-100 mg/m^2/day of iv hydrocortisone (6 hourly injections) or 2-3 mg/m^2/hour (continuous iv) should be administered during

the first 24 hours and reduced to 50 mg/m^2 during the following day. Fludrocortisone is not required acutely due to the mineralocorticoid effect of high hydrocortisone dosage. Depending on the clinical state, the hydrocortisone should be switched to an oral regimen, starting with 30-50 mg/m^2/day and reduced gradually to maintenance doses. In this moment, fludrocortisone 0.05-0.1 mg/day should be provided (2,35,39-41). As the stress doses are empirical and not based on controlled clinical trials, the recommended glucocorticoid stress doses vary in pediatric patients. Further studies are needed to define the ideal stress dose in pediatric patients, because both under- and overdoses are harmful to patients.

The factor must be investigated and treated in all patients with AC. Sodium and potassium levels should be monitored and iv dextrose should be administered in cases of hypoglycemia, more common in children. Antibiotic therapy, admission to an intensive care unit, and administration of a low dose of heparin should be considered (26,27,37).

The AC approach is often effective, with patient recovery within 24 hours. If recovery does not occur, two possibilities must be considered: Either there is another cause for the patient's serious impairment or the patient has reached "a point of no return" when even optimum care will no longer avert death from AC (27).

Prevention of AC

Adrenal insufficiency patients, their families, and their caregivers should be constantly informed that adherence to continuous glucocorticoid therapy throughout life and adequate adjustment of glucocorticoid doses in stressful situations are essential to avert AC and death. Fever > 38 °C (100.4 °F), intercurrent illness with emesis, prolonged or voluminous diarrhea, infectious disease requiring antibiotics, acute trauma requiring medical intervention (e.g., fracture), and anesthesia-associated surgical procedures are considered stressful conditions. No randomized controlled studies have evaluated glucocorticoid dose requirements during stressful situations in either adults or children. Therefore, glucocorticoid doses are typically based on the general acceptance that cortisol levels rise 2 to 3 times during maximally stressful situations and on the severity and duration of the stressor or illness (2,27,35).

During fever, oral hydrocortisone replacement doses should be doubled (>38 °C) or tripled (>39 °C) until recovery (usually 2 to 3 days), and the consumption of electrolyte-containing fluids should be encouraged as tolerated. As soon as recovery occurs, the doses should be rapidly (within 1 to 2 days) reduced to the standard replacement doses. If oral medication is not tolerated (vomiting, diarrhea, or trauma), 100 mg of hydrocortisone for adults (50 mg/m^2 for children), IV, IM, or subcutaneous (SC), should be either self-administered or administered by a physician. It may be necessary to repeat this dose. Health care professionals should be involved early for clinical assessment. Mineralocorticoid replacement is not required if the hydrocortisone dose exceeds 50 mg/24 h due to the mineralocorticoid effect of high hydrocortisone dosage (Table 2) (2,27,33,42).

Table 2. Stress doses of hydrocortisone to prevent and/or treat adrenal crisis

Clinical Condition	Treatment
Fever and illness – home management	Hydrocortisone replacement doses doubled (>38 °C) or tripled (>39 °C) until recovery and increased consumption of electrolyte-containing fluids as tolerated
Oral intake prevented by vomiting or trauma	Adults: 100 mg IM or SC hydrocortisone (children: 50 mg/m^2)
Minor to moderate surgical stress	Doubled or tripled hydrocortisone oral doses or 25-75 mg/24 h iv hydrocortisone (usually 1 to 2 d) in adults (50 mg/m^2 in children)
Major surgery with general anesthesia, trauma, delivery, or disease that requires intensive care	100 mg IV hydrocortisone followed by 200 mg/d hydrocortisone as a continuous infusion for 24 h or every 6 h, reduced to 100 mg/d hydrocortisone the following day. Children: 50-100 mg/m^2 hydrocortisone bolus at the induction of anesthesia, followed by 50-100 mg/m^2/d hydrocortisone divided q 6 h. Decrease to 50 mg/m^2/d hydrocortisone on the second day and 25 mg/m^2/d on the third day.

Adapted from: Allolio (27), Miller et al. (35) and Shulman et al. (40).

Concerning surgical stress, it is estimated that adults secrete 75-100 mg of cortisol/day in response to major surgery and 50 mg/d in response to minor surgery (42). Prior to minor or moderate surgical stress, doubled or tripled hydrocortisone replacement oral doses or 25-75 mg/24 h IV hydrocortisone is recommended for adults (usually 1 to 2 days) and 50 mg/m^2/24 h for children during the procedure. Prior to major surgery with general anesthesia or during delivery (4 cm cervix dilation and/or contractions every 5 min for the last hour), trauma, or disease that requires intensive care, the parenteral

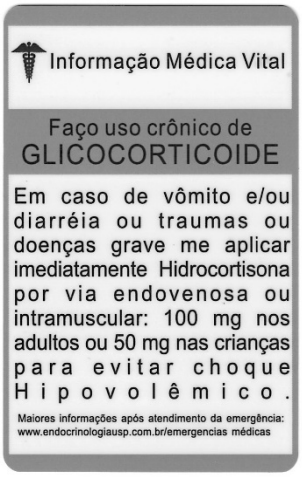
hydrocortisone management should be performed as in AC, for both adults and children (Table 2) (2,40). In SW CAH children, we suggest the oral administration of the usual mineralocorticoid doses around 4 hours before the start of surgery (unpublished data).

Patients' education about AC prevention

AC in patients with known PAI and CAH is best prevented by educating patients and caregivers on the precocious identification of AC and the glucocorticoid dose adjustment during stressful situations (27).

However, it has been shown that a high percentage of patients (46%) were not sufficiently skilled in steroid management with physical stress (43). The most effective strategy to check if the patients are regularly on such therapy is by asking them if they are aware of how important the medication is, how it must be taken daily, and how it should be taken in different stressful situations (Table 2). If the patient fails, information should be provided again, and all doubts should be cleared.

Besides that, an AC prevention card, with a medical alert to inform patients and health professionals about the acute hydrocortisone injection (IM or IV) in stressful situations, should be offered to the patients and caregivers (Figure 1) (37,38,44). The patients should carry the card with them constantly, which should be checked by the physician at every medical appointment. This card should be available for all patients with adrenal insufficiency to avoid hypovolemic shock and death. The cost is reasonable (around US$ 40.00 for 1,000 cards in Brazil) and should ideally be supported by the hospital.



Vital medical information

I use chronic glucocorticoid. In case of vomiting and/or diarrhea or trauma, give me immediately intravenous or intramuscular hydrocortisone: 100 mg in adults or 50 mg in children to avoid hypovolemic shock.

Figure 1. Adrenal crisis treatment card.

In addition, every patient should carry an emergency glucocorticoid injection kit consisting of 100 mg of hydrocortisone for parenteral or SC administration. This kit urges the first parenteral administration of glucocorticoids even before the patient can get in touch with a doctor or reach an emergency service (2). In Brazil, this kit is not commercially available yet, and efforts are being made to enable pharmaceutical companies to provide it here, because injectable hydrocortisone is only available in hospitals.

In conclusion, thus, there is an increased mortality among PAI and CAH patients linked to episodes of AC, which occur frequently during infections and stress conditions. If the AC is promptly identified and managed, morbidity and mortality is reduced. Unfortunately, as the clinical features of imminent AC are often nonspecific, the glucocorticoid stress dose is frequently delayed. Effective prevention of AC remains a major challenge in the care of these patients. Patients' and caregivers' education, the emergency card, and the glucocorticoid kit are essential to preventing AC and reducing the morbimortality.

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Thyroid collision tumor containing oncocytic carcinoma, classical and hobnail variants of papillary carcinoma and areas of poorly differentiated carcinoma

Marcos Tadashi Kakitani Toyoshima[1]
https://orcid.org/0000-0002-9146-4606

Regina Barros Domingues[2]
https://orcid.org/0000-0003-4564-9957

Ibere Cauduro Soares[2]
https://orcid.org/0000-0003-3830-4686

Debora Lucia Seguro Danilovic[1]
https://orcid.org/0000-0002-4058-8027

Larissa Costa Amorim[3]
https://orcid.org/0000-0003-0433-1299

Edla R. C. Cavalcante[3]
https://orcid.org/0000-0003-4663-5100

Fernanda F. Antonacio[3]
https://orcid.org/0000-0002-7157-5181

Felipe Santa Rosa Roitberg[3]
https://orcid.org/0000-0003-2546-543X

Ana Oliveira Hoff[1]
https://orcid.org/0000-0002-7058-6321

[1] Serviço de Onco-Endocrinologia, Instituto do Câncer do Estado de São Paulo Octavio Frias de Oliveira, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil
[2] Departamento de Patologia, Instituto do Câncer do Estado de São Paulo Octavio Frias de Oliveira, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil
[3] Departamento de Oncologia Clínica, Instituto do Câncer do Estado de São Paulo Octavio Frias de Oliveira, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil

SUMMARY

Collision tumors are rare and may comprise components with different behavior, treatments, and prognosis. We report an unprecedented case of aggressive thyroid collision tumor containing widely invasive oncocytic carcinoma (OC), classical and hobnail (HPTC) variants of papillary carcinoma, and poorly differentiated carcinoma (PDTC). The patient underwent total thyroidectomy, radioactive iodine therapy, and within months progressed with local recurrence, and pulmonary metastases requiring neck dissection, external radiotherapy and systemic treatment with sorafenib. The rapid progression, dedifferentiated metastatic lesions, and failure to treatments resulted in the patient´s death. The great variety of histological types and the evolution of this case were a challenge for the management of metastatic disease. Widely invasive OC, HPTC and PDTC are considered to have a worse prognosis. HPTC has never been reported as a component of a collision tumor. HPTC and PDTC should call attention to a possible higher-grade transformation. Arch Endocrinol Metab. 2021;65(4):495-9

Correspondence to:
Marcos Tadashi Kakitani Toyoshima
Serviço de Oncoendocrinologia do Instituto do Câncer do Estado de São Paulo
Av. Dr. Arnaldo, 251, Cerqueira César
01246-000 – São Paulo, SP, Brasil
marcos.tadashi@hc.fm.usp.br

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INTRODUCTION

Differentiated thyroid carcinomas (DTC) are the most prevalent malignant endocrine tumors (1). Papillary (PTC) and follicular (FTC) thyroid carcinomas are the most prevalent DTC. In general, thyroid cancer has a good prognosis with 10-year overall survival rates for patients with PTC and FTC of 93% and 85%, respectively (2). However, some histopathological variants of follicular cell-derived thyroid cancer, such as (i) tall cell, (ii) columnar cell and (iii) hobnail variants of PTC; (iv) widely invasive FTC; and (v) poorly-differentiated carcinoma (PDTC) are associated with more unfavorable outcomes, including increased risk of tumor-related death (3).

Oncocytes, also known as Hürthle cells, are large, polygonal, epithelial cells with an acidophilic cytoplasm, containing a great number of mitochondria, large hyperchromatic nucleus, and prominent nucleolus. Oncocytes are associated with benign conditions, as lymphocytic thyroiditis, Graves disease, and hyperplastic nodules in multinodular goiters. Malignant diseases that are associated with oncocytes include an oncocytic

variant of PTC and minimally invasive or widely invasive oncocytic carcinoma (OC) (4,5). OC accounts for about 5% of differentiated thyroid cancers (6,7) and was previously considered to be a variant of FTC; however, after thorough genomic analysis, it has been considered distinct from FTC (8). In addition, OC has a more aggressive behavior and is less avid to radioactive iodine compared to FTC (6). The metastatic spread of OC is mainly hematogenous, and therefore lymph node metastases are less common than PTC. The risk of recurrence of widely invasive OC is 73% (5) and the estimated 5- and 10-year disease-specific survival rates of metastatic OC are 81% and 60%, respectively (7).

In 2004, a Japanese study (9) showed that the loss of the cell polarity (hobnail appearance) could be a characteristic of poor cell differentiation and an increased risk of tumor recurrence in PTC. However, the hobnail variant of PTC (HPTC) was only described in 2009 (10), and since then, more than 100 HPTC cases have been described (11-13). Currently, HPTC is a recognized aggressive variant of PTC. The loss of polarity/cohesiveness with hobnail features in ≥30% of tumor cells is one of the criteria for the diagnosis of HPTC (11). PTC with hobnail/micropapillary characteristics (<30% of tumor cells with hobnail features) appears to be more aggressive than classic papillary carcinoma (14).

PDTC is an aggressive thyroid tumor, characterized by a partial loss of thyroid differentiation. Its morphological and behavioral characteristics are intermediate between DTC and anaplastic carcinoma. PDTC causes locally invasive cervical disease in more than 50%, metastasis to lymph nodes (LNs) in 50% to 85%, and distant metastasis in up to 85% of cases. Five-year disease-specific survival for PDTC patients has been reported at 66% (15).

Collision tumors, defined as two or more neoplasms coexisting in one anatomical site with distinct histology, are rare (16). Thyroid collision tumors are rare, and just over 40 case reports have been described (16-23).

We report an unprecedented case of aggressive thyroid collision tumor containing OC, classical and hobnail variants of PTC, and areas with PDTC.

CASE REPORT

A 63-year-old woman with a long-standing history of thyroid nodules was referred to our hospital. She knew the diagnosis of thyroid nodules for 25 years. Thyroid ultrasonography (US) showed heterogeneous nodules with gross calcifications in the left lobe of the thyroid, the largest being 4.9 x 6.4 x 3.5 cm. A fine-needle aspiration (FNA) biopsy of this nodule revealed a follicular neoplasm with oncocytes (Bethesda category IV). The patient refused surgical treatment for 4 years, when she developed pain and dysphagia. She was then submitted to total thyroidectomy, and the histopathologic report depicted three components in the left lobe of the thyroid: widely invasive OC, measuring approximately 6.0 cm in the longest axis, in addition, classic variant PTC (CVPTC), but with hobnail component and foci of poorly-differentiated carcinoma (Figure 1, Figure 2 and Figure 3). The CVPTC measured 0.8 cm and the set of hobnail and poorly differentiated components measured 1.8 cm in the longest axis. The hobnail component of papillary carcinoma was characterized by the presence of papillary and micropapillary structures lined by cells with decreased nucleus-cytoplasm ratio, loss of cohesiveness, eosinophilic cytoplasm, apical nuclei and with evident nucleoli. The poorly differentiated component was characterized by the presence of solid blocks of cells, but without nuclear characteristics of papillary carcinoma, exhibiting intense atypia, vesicular nuclei with evident nucleoli, presence of mitoses (3 mitoses in 10 high magnification fields) and necrosis. Surgical margins were free of disease, and there was no extrathyroidal extension, angiolymphatic invasion, nor perineural invasion. Adenomatous goiter with areas of follicular hyperplasia, associated with chronic lymphocytic thyroiditis, was found around these tumors. The 7th American Joint Committee and International Cancer Control and the American Joint Committee on Cancer (AJCC/UICC) TNM (tumor-node-metastasis) thyroid cancer staging was defined as pT3pNxpMx. The immunohistochemical profile (IHC) revealed focal thyroglobulin staining in all tumoral components (OC, CVPTC, HPTC and PDTC); TTF-1 positive in OC, and inconclusive in PTC; PAX8 was focal positive in OC (Figure 4), and inconclusive in CVPTC; p53 positive staining was observed in OC, HPTC and PDTC, and inconclusive in CVPTC.

BRAF mutational analysis by gene sequencing was performed on samples of all components of thyroid carcinoma, but no mutations were found.

The patient underwent radioactive iodine therapy (RAI) with ^{131}I with 206 mCi (7622Mbq) upon thyroid hormone withdrawal. Serum TSH and thyroglobulin (TG) before RAI were 91.08 mcU/mL and

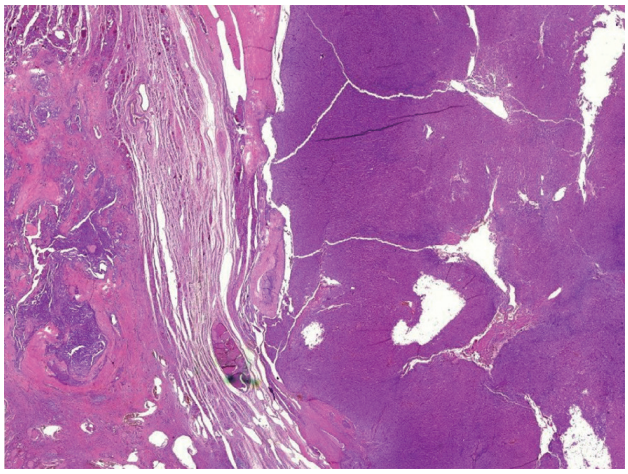

Figure 1. Photomicrograph of the surgical specimen showing stain, showing two components of the collision tumor. On the right side of the figure, oncocytic carcinoma with capsular invasion and on the left side, classic variant of papillary thyroid carcinoma (H&E, 25X).

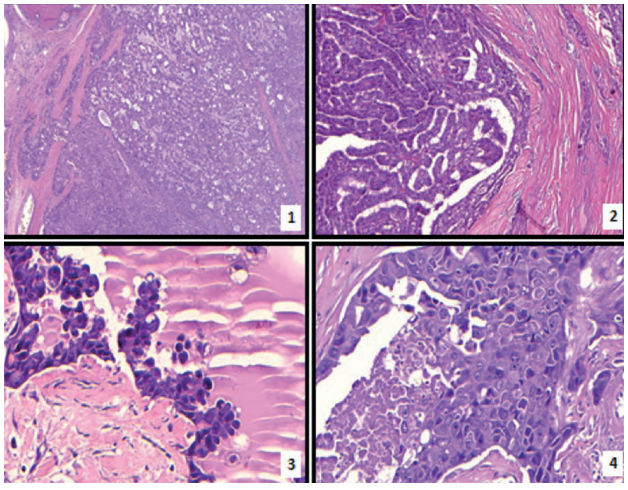

Figure 2. Photomicrographs of the tumor. Histopathological features of the four components of the collision tumor: (**1**) Oncocytic carcinoma with capsular invasion (H&E, 40x); (**2**) Conventional (classic) papillary carcinoma (H&E, 100x); (**3**) Hobnail variant of papillary thyroid carcinoma (H&E, 400x); (**4**) Poorly differentiated thyroid carcinoma (H&E, 400x).

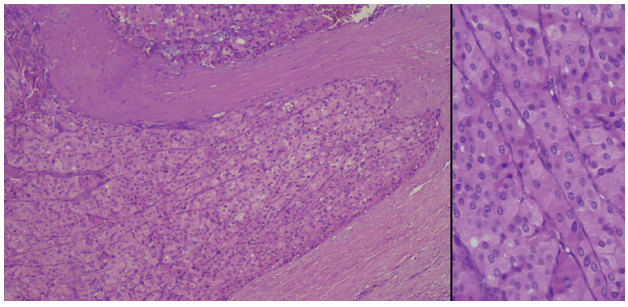

Figure 3. Higher magnification of oncocytic carcinoma with capsular invasion (H&E, 100x, 400x at right).

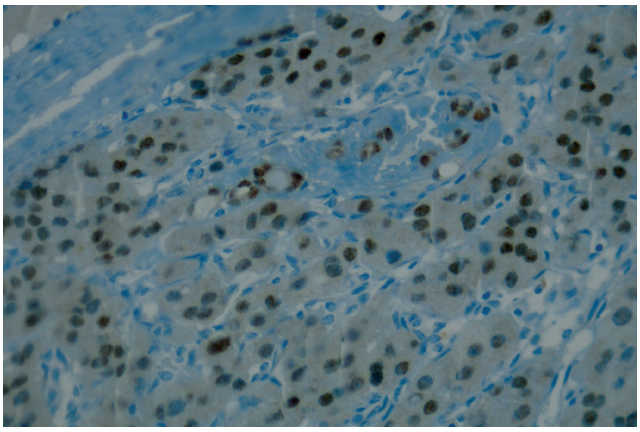

Figure 4. PAX8 positivity at oncocytic carcinoma (immunohistochemistry, 400x).

6.0 ng/mL, respectively. The post-treatment ^{131}I whole-body scan showed uptake in thyroid bed and possible central compartment neck LNs. The radioactive iodine uptake was 1.5%.

Six months later at a follow-up visit, serum TG and anti-TG antibodies were undetectable, neck US revealed heterogeneous LNs ranging from 1.3 to 1.6 cm in level IV bilaterally which were biopsied; cytology from both LNs was consistent with metastatic carcinoma, with morphological aspect similar to the PDTC, despite an undetectable TG from both LNs aspirate. The IHC panel showed positive focal immunoexpression for TTF-1, and negative for thyroglobulin and PAX8.

In the face of a recurrence from a poorly-differentiated tumor, a positron emission tomography-computed tomography (PET-CT) with fluorine-18 fluorodeoxyglucose (^{18}F-FDG) was obtained showing not only both LNs identified by the US (SUVmax 16.5%) but also several pulmonary nodules, some cavitating, the largest with 2.2 cm in its largest diameter (SUVmax 5.6%).

Lung biopsy revealed the presence of papillary pattern adenocarcinoma, with positive immunoexpression only for cytokeratin 7 (CK7), inconclusive for PAX8 and P53 and negative for thyroglobulin and TTF-1. Other markers indicative of origin in the breast, lower digestive tract or lungs were negative. The immunoexpression of PAX8 and p53 proved to be inconclusive.

Because of the significant progression of local disease, she underwent bilateral LN dissection (levels II-V), which path report revealed metastasis in 6 out of 49 LNs with extensive extracapsular invasion involving fibroadipose tissue and skeletal muscle and a 5-cm soft tissue metastasis

infiltrating skeletal muscle from levels II-IV on the left. IHC was consistent with a poorly-differentiated carcinoma of unknown primary source (p16, p63, CK7 and GATA-3 positive, c-erb-B2/HER-2, p53, WT1, BRST2, Cdx2, CK20 cytokeratin, PAX8, estrogen receptor, thyroglobulin, TTF-1 all negative). Within a month from this surgical procedure, she developed acute respiratory failure requiring emergency tracheostomy. Staging workup (computed tomography scan of the head, neck, and chest and ^{18}F-FDG PET-CT) showed evidence of local recurrence, enlargement of pulmonary nodules, and new liver metastases. She underwent external-beam radiotherapy to the cervical area (cumulative dose of 20Gy) and initiated treatment with sorafenib 400mg twice a day with good tolerance but with no therapeutic response. She died two months later with the rapid progression of the pulmonary metastases.

DISCUSSION

We report a case of a patient with a collision tumor containing widely invasive OC, classical and hobnail variants of PTC, and areas with PDTC. Collision tumors can contain components with different aggressiveness, treatments, and prognosis, challenging their management (24). Widely invasive OC, HPTC, and PDTC are some of the thyroid cancers that are considered to have a worse prognosis among DTC (3).

Several hypotheses have been suggested as mechanisms for collision tumors: (i) a simple coincidence, (ii) one tumor predisposing to the other, (iii) a carcinogenic factor predisposing the tumors involved, or (iv) tumors that derive from stem cell remnants (17). Most reports of thyroid collision tumors consist of papillary and medullary thyroid carcinomas (16).

Our literature review found five case reports of collision tumors containing PTC and OC (19,25-27). As in our case report, three works also reported association with Hashimoto's thyroiditis (19,26,27). However, the relationship between Hashimoto's thyroiditis and the pathogenesis of thyroid cancer, especially PTC, remains controversial (28).

HPTC is a rare and quite aggressive variant of PTC and has not yet been described as a component of a collision tumor of the thyroid. The hobnail features have been reported in cases of poorly-differentiated and anaplastic thyroid carcinoma. The presence of these features should alert to a possible higher-grade transformation (29).

PDTC is associated with an increased risk of metastasis and tumor-related death (3,15). The presence of PDTC in the collision tumor is a sign of aggressiveness, poor prognosis and likely transformation of the more differentiated PTC (24).

The somatic mutation burden is relatively low in thyroid tumors, making it easier to understand their pathogenesis. Driver mutations are identified in more than 90% of thyroid cancers, and passenger mutations that can modify the biological behavior of the tumor can occur in many tumors (30). The main genes with mutations in the HPTC are *BRAF* and *TP53*. *RET/PTC1* rearrangements and in the *TERT* promoter mutations have also been reported (13). The most common mutations in PDTC are *TERT*, *BRAF*, *RAS*, and *TP53* mutations (15). The sample size of the cancer subtypes was limited for the genetic analysis of our patient, permitting only the analysis of the *BRAF* gene, which resulted without mutations. Genetic and epigenetic changes are involved in the initiation, progression, and dedifferentiation of cancers, including thyroid cancer. *BRAF* and *RAS* mutations are more involved in an earlier event of the tumorigenesis, and mutations in the *TP53* and *CTNNB1* genes, for example, in later events (1). This case called our attention to the rapid and severe progression of the disease. It led us to raise the hypothesis of the dedifferentiation of the metastases (1) and the successive transformations of the tumor, possibly from classical variant PTC to HPTC and from HPTC to PDTC. Unfortunately, a more thorough genetic analysis of the tumor was not possible. However, the IHC profile showing positivity for p53 in HPTC, and in poorly-differentiated carcinoma components suggest gaining of poor prognostic mutations. The lack of response to therapy and the short survival time observed in this case was similar to the expected survival observed in anaplastic thyroid carcinoma. However, it was not detected in any of the performed biopsies.

To conclude, this is a very rare case report of a thyroid collision tumor involving several histological patterns of carcinoma, including PTC with hobnail characteristics and possible progression of the component of PDTC with a very aggressive evolution.

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Repetitive stress fracture: a warning sign of genetic susceptibility to fracture? A case report of a heterozygous variant in *SERPINF1*

[1] Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil
[2] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Mariana Lima Mascarenhas Moreira[1]
https://orcid.org/0000-0001-8003-1293

Iana Mizumukai de Araújo[1]
https://orcid.org/0000-0003-0903-4787

Greice Andreotti de Molfetta[2]
https://orcid.org/0000-0003-0946-5704

Wilson Araújo Silva Jr.[2]
https://orcid.org/0000-0001-9364-2886

Francisco José Albuquerque de Paula[1]
https://orcid.org/0000-0003-1262-3486

SUMMARY

The occurrence of fractures in young individuals is frequently overlooked by physicians, especially when associated with exercise or trauma. Nevertheless, multiple fractures should always be investigated since underlying conditions can predispose to such events. We describe here the case of a young, healthy woman who sustained multiple fractures in the lower limbs, which were initially considered to be "stress fractures". Further investigation, including a panel of genes associated with osteogenesis imperfecta, revealed that the patient is a heterozygous carrier of a *SERPINF1* variant. According to criteria recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, this variant is classified as likely benign (PM2, PP3, PP4, BP1, and BP4). The patient's mother and brother were also asymptomatic carriers of the variant and had sustained previous minor fractures. The patient had normal biochemical profile and bone density. This condition has been rarely described and is not associated with low bone mineral density or altered bone turnover markers. This case highlights the importance of investigating multiple fractures in young patients who are otherwise healthy since these may be a warning sign of rare genetic conditions associated with fragility fractures. Arch Endocrinol Metab. 2021;65(4):500-4

Correspondence to:
Francisco J. A. de Paula
Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo
Avenida Bandeirantes, 3.900, Monte Alegre
14049-900 – Ribeirão Preto, SP, Brasil
fjpaula@fmrp.usp.br

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INTRODUCTION

The term "stress fracture" encompasses two distinct processes. One is due to excessive and repetitive load on the skeleton, known as "fatigue fracture", which was first described almost two centuries ago in Prussian soldiers (1). The other one occurs under normal load in weak bone and is known as "insufficiency fracture". Fatigue fractures occur typically in young subjects, most frequently women, and in approximately 75% of the cases affect the tibia, tarsal bones, and metatarsals. We report here the case of a young woman who sustained, in conditions of moderate exercise, four fractures with mixed components of fatigue and insufficiency fractures.

Genetics is a major determinant of bone strength and influences both bone mass and microstructural properties. Similar to other complex diseases, primary osteoporosis has a genetic background associated with multiple genetic variants across several genes, each having small contributions to bone phenotype. On the other hand, rare genetic diseases, in which variants in a single gene can cause severe bone fragility, offer a window of opportunity for unveiling molecular mechanisms directly involved in fracture susceptibility and development of new therapeutic drugs for osteoporosis. Repeated episodes of fragility fractures in young individuals are natural signs for researchers and clinicians pursuing advances in the

science of the mechanisms, diagnosis, and therapy of osteoporosis. The last decades have led to the identification of several genetic defects driving bone fragility disorders. For example, variants in *LRP5* (2) and *WNT1* (3), which in the homozygous state cause the osteoporosis-pseudoglioma syndrome and a severe form of osteogenesis imperfecta (OI), respectively, are associated with early-onset osteoporosis in heterozygous conditions. The recessive homozygous variants in the *SERPINF1* gene were originally described as the cause of type VI OI, a moderate to severe disease with a progressive course and poor response to bisphosphonates. However, previous reports have shown heterozygous carriers of the *SERPINF1* variant without abnormal areal bone density, volumetric bone density, or markers of bone metabolism (4), suggesting the absence of skeletal disorders, although data on fractures was not presented.

We report here a case of a phenotypically healthy young woman whose clinical manifestations – four sequential fractures elicited by mild or at the most moderate exercise – combined components of fatigue and insufficiency fractures. The present study points out the mild impairment in bone strength among individuals exhibiting heterozygous *SERPINF1* variants. We believe that this case illustrates the role of research in identifying new genes associated with bone fragility.

CASE REPORT

This case report was approved by the institutional review board of the University Hospital of the Ribeirao Preto Medical School at USP (CAAE: 38807320.5.0000.5440). The patient provided written informed consent.

A 33-year-old woman attended our outpatient clinic with a history of four previous fractures that had occurred in mild or moderate mechanical stress situations after she started regularly attending a gym. Her first fracture occurred 4 years before the medical consultation, in the left distal tibia, after a 20-minute run on a treadmill, and was confirmed by magnetic resonance imaging (MRI) (Figure 1). She sustained two subsequent fractures in the same region during light runs, 3 and 7 months after the first fracture. The fourth fracture affected the right distal tibia and occurred while the patient practiced with a jump rope. No other fractures were described. She practiced running mostly

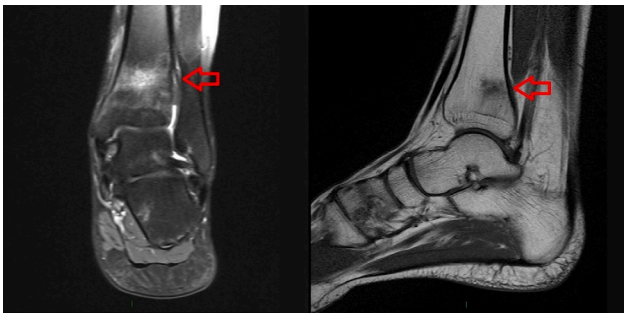

Figure 1. Magnetic resonance imaging showing coronal T2 and sagittal T1 views of the patient's fracture in the left distal tibia.

indoors and after warming up. She had no history of eating disorders. She had mild dyslipidemia, which was treated with rosuvastatin 10 mg, but had no family history of dyslipidemia. She was taking vitamin D3 2000 IU/daily since the first fracture but had sufficient vitamin D levels. She was otherwise healthy and had no previous clinical conditions.

Her menses started at age 13 and were always regular. At the time of the first appointment, she was nulliparous and was not using contraceptive pills. She had no previous use of glucocorticoids. She denied smoking, had a low intake of alcohol, consumed a low amount of dairy products, and had reduced sun exposure. Her parents were unrelated. Her mother had a medical history of osteopenia and had sustained a forearm fracture in her youth; her father was healthy. Her brother was healthy but also had a history of fractures associated with the practice of radical sports.

The patient was 164.5 cm tall and weighed 67.1 kg. Her body mass index was 24.79 kg/m^2 at the time of the first appointment. Her sclera was slightly darkened, and she had no visible bone deformities or disorders of tooth development. No abnormality was detected in her clinical examination. She had no pathological features in her lower limbs, her legs were aligned, and her feet had a normal arch.

The patient's biochemical tests revealed levels of total serum calcium of 9.6 mg/dL (reference values [RV] 8.5-10.5 mg/dL), 25-hydroxyvitamin D (25(OH)D) of 30 ng/mL (RV 20-50 ng/mL), phosphorous 3.4 mg/dL (RV 2.5-5.0 mg/dL), alkaline phosphatase 60 U/L (RV 35-104 U/L), serum creatinine 0.7 mg/dL (RV 0.7-1.5 mg/dL), TSH 2.4 mIU/L (RV 0.4-4.5 mIU/L), PTH 31 pg/mL (RV 10-65 pg/mL), prolactin 6.5 µ/L (RV < 31 µ/L), LH 9.0 IU/L (RV < 12 IU/L), total cholesterol 260 mg/dL, LDL-cholesterol 174 mg/dL, triglycerides 298 mg/dL, and normal complete blood count.

The initial bone densitometry (Hologic Horizon, Waltham, MA, USA) report indicated: lumbar spine (L1-L4) 0.945 g/cm², Z-score -0.5; total hip 0.845 g/cm², Z-score -0.8; femoral neck 0.688 g/cm², Z-score -1.5. Her most recent densitometry, 5 years after the first, showed: L1-L4 0.976 g/cm², Z-score -0.6; total hip 0.806 g/cm², Z-score -1.0; femoral neck 0.652, Z-score -1.6.

Since the patient had recurrent fragility fractures, a next-generation sequencing (NGS) panel for bone fragility disease was performed at our Genomic Medicine Center through sequencing of the following 13 genes: *COL1A1*, *COL1A2*, *SERPINH1*, *FKBP10*, *SP7*, *BMP1*, *TMEM38B*, *WNT1*, *IFITM5*, *SERPINF1*, *CRTAP*, *LEPRE1*, and *PPIB*. DNA was extracted from blood samples from the patient, her mother, and her brother using the Super Quik-Gene-Rapid DNA Isolation kit (Promega Corp., Madison, WI, USA). Sequencing was performed using the Ion Personal Genome Machine (PGM; Life Technologies, Darmstadt, Germany) as per the manufacturer's instructions with the 200-bp single-end run configuration. The analyses of raw data mapping (against GRCh37/hg19), calling of variants, and variants annotation were processed using the Torrent Suite Software, version 4.4.2, the Torrent Variant Caller Plugin, and the Ion Reporter Software, version 5.16.0.2, respectively (all by Life Technologies).

We identified a heterozygous variant – p.Ala369Val (c.1106C>T) rs368630571 – in the *SERPINF1* gene that had no description of clinical significance in the ClinVar platform but was likely associated with slight bone fragility. The same variant was identified in the other family members (Figures 2a and 2b). This is a missense variant, located in exon 8. It was defined as potentially pathogenic by the UMD-predictor (score 69) and CADD (score 16.17) software applications (5,6) and had a global allele frequency of 0.0001273%, according to the Genome Aggregation Database (gnomAD) (7). According to the recommendation criteria by the American College of Medical Genetics

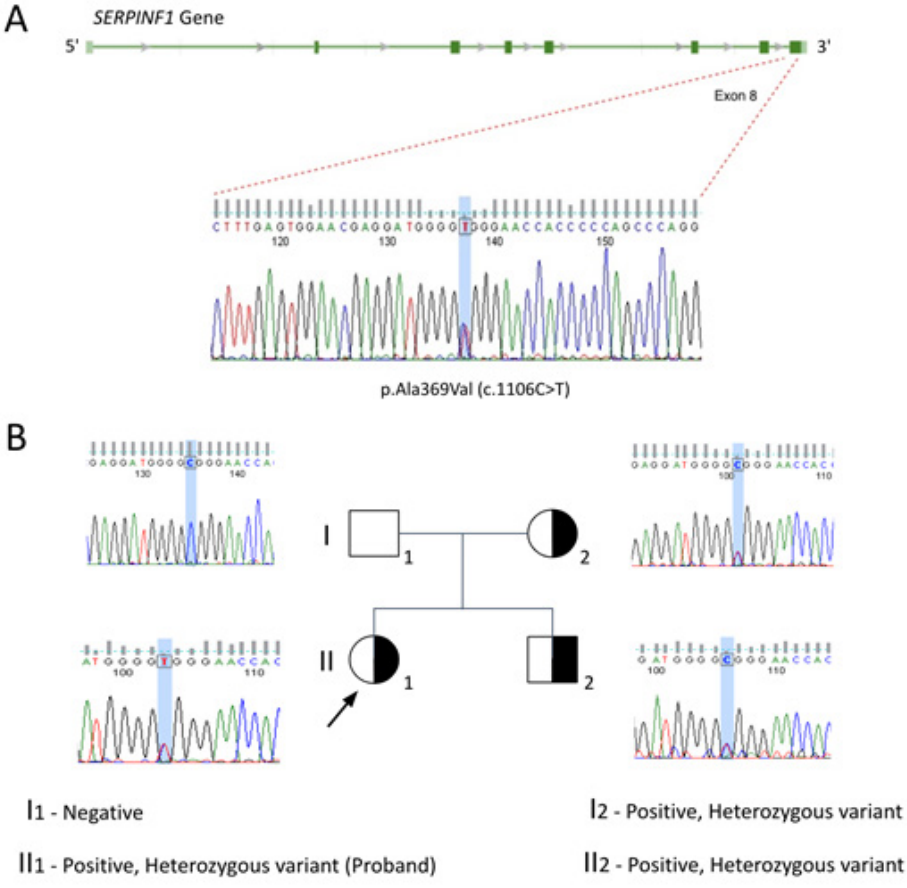

Figure 2. (**A**) Heterozygous variant in the *SERPINF1* gene found in a female patient with multiple fractures. (**B**) Family genogram of the *SERPINF1* variant affecting three family members, including the patient, her mother, and a brother. The validation of the variant and the analysis of family segregation were performed after amplifying and sequencing the target region by the Sanger method following the manufacturer's recommendations (Applied Biosystems Genetic Analyzer, ThermoFisher Scientific, Waltham, MA, USA).

and Genomics and the Association for Molecular Pathology (8), this variant is classified as likely benign (PM2, PP3, PP4, BP1, and BP4). This classification was supported by the Genetic Variant Interpretation Tool (9) and VarSome (10) platforms. The classification of the variant followed the recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (8). The variant was not identified in the ABraOM database (11). *SERPINF1* variants are associated with type VI OI. Since this is an autosomal recessive disease, individuals with heterozygous variants are expected to be phenotypically normal.

DISCUSSION

The most well-known forms of OI with autosomal dominant inheritance are the classical types I-IV, which are associated with variants in the *COL1A1* and *COL1A2* genes. More recently, variants in collagen-related genes have been described as causing other forms of OI. The *SERPINF1* is included in this newer group of autosomal recessive inheritance and expresses a collagen chaperoning protein. The homozygous variant in this gene causes OI independently from collagen type-I synthesis. *SERPINF1* encodes the pigment epithelium-derived factor (PEDF), which appears to have a high affinity to collagens of the extracellular matrix (12) and acts as an inhibitor of bone resorption (13). *SERPINF1* is described as a gene associated with rare monogenic diseases that have an impact on bone mass and strength (14), including in the Brazilian population (15). In homozygosity, bone fragility is well recognized, but in heterozygosity, there is still a gap in our knowledge regarding the occurrence of alterations. The study of candidate alleles for bone fragility is still growing, and the report of single individuals or families with variants can help fill this gap.

In a previous study, Al-Jallad and cols. (2014) described that heterozygous *SERPINF1* variant carriers had no detectable abnormalities in bone mass or adipose tissue distribution (4). Compared with non-carriers, carriers of this variant had similar body mass indexes and levels of bone turnover markers and no significant differences in bone mineral density, with the only exception for levels of PEDF, which were significantly lower in the carrier group. Although this protein has several potential functions, its deficiency apparently expresses as a specific skeletal phenotype with no other clinical manifestations.

Our patient also had a normal clinical phenotype, no biochemical abnormalities, and bone mineral density that was adequate for her age. However, she had a clinically significant outcome of four fractures in circumstances that combined stress and fatigue fracture. Measurement of PEDF levels was not done in the present case. Al-Jallad and cols. (2014) mentioned that the clinical effect of variants in only one *SERPINF1* gene (in heterozygosis) is lower than the one observed in genes *WNT1* and *LRP5*, which in heterozygosis lead to osteoporosis, although there was no information from the investigated individuals on activities that required physical effort (4). It is important to point out that *LRP5* and *WNT1* play a clear role in the *WNT* signaling pathway, while the *PEDF* mechanisms on bone fragility remain to be determined.

One concern raised during the investigation of this patient was the differential diagnosis with stress fractures in female athletes, since women are at increased risk for this type of fracture (16). The female athlete triad comprises caloric restriction, amenorrhea, and low bone mass. None of these features were part of the clinical profile of our patient. Moreover, our patient did not meet the criteria for this condition since her pain onset was acute and not insidious, as typically occurring in stress fractures (17). She had no risk factors for low bone density – such as smoking, high alcohol intake, rheumatoid arthritis, or glucocorticoid use – or a low Z-score on bone densitometry. Despite her low consumption of dairy products, this scenario is compatible with the one found in the Brazilian population. According to the International Osteoporosis Foundation (IOF), the mean daily calcium intake in the Brazilian population is 505 mg; thus, we do not attribute her history of fractures to calcium intake alone. Altogether, the clinical presentation of our patient seems to blend the two types of stress fracture, meaning that the fragility fracture occurred with mechanical stress insufficient to provoke a fatigue fracture in a young woman. These circumstances signaled an underlying cause for the recurrent fractures. Even though the variant is classified as likely benign, we hypothesize that we are facing a case of haploinsufficiency, which occurs when the normal phenotype requires the protein product of both alleles, and a 50% reduction in protein production results in an abnormal phenotype (18,19).

Using genomic, functional, and evolutionary characteristics, a group of researchers put together a predictive model of haploinsufficient genes and compiled a list of 12,443 genes (20). Interestingly, the *SERPINF1* gene is one of the predicted genes on the list. Its haploinsufficient profile still needs to be experimentally validated. However, depending on the clinical profile, individuals who are heterozygous for the *SERPINF1* gene may still exhibit subtle abnormalities, which should be kept in mind when patients are evaluated.

In conclusion, underneath repeated fragility fractures in young individuals usually lies a predisposing secondary condition, which may not be clinically or biochemically detectable. The findings of the present study show, for the first time in the literature, that a "stress fracture" can be a clinical manifestation in a patient harboring a heterozygote variant in *SERPINF1*. The present study highlights the relevance of molecular investigation in special cases of osteoporosis, a neglected disorder, even when repeated fractures are part of the clinical scenario. A larger number of studies and consistent investigations are nonetheless needed to elucidate the bone phenotype in carriers of *SERPINF1* variants and their association with bone fragility. When possible, a thorough investigation is recommended since it can further contribute to the identification of candidate genes for bone fragility.

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Use of aromatase inhibitors in patients with breast cancer is associated with deterioration of bone microarchitecture and density

Frederico Arthur Pereira Nunes[1,2]
https://orcid.org/0000-0002-1220-417X

Maria Lucia Fleiuss de Farias[1]
https://orcid.org/0000-0003-0466-3250

Felipe Peres Oliveira[1]
https://orcid.org/0000-0002-4689-7242

Leonardo Vieira Neto[1]
https://orcid.org/0000-0002-1595-3985

Luis Felipe Cardoso Lima[3]
https://orcid.org/0000-0002-5481-1090

Francisco de Paula Paranhos Neto[1]
https://orcid.org/0000-0001-6661-0993

Laura Maria Carvalho de Mendonça[4]
https://orcid.org/0000-0003-2129-3546

Miguel Madeira[1]
https://orcid.org/0000-0001-6752-2880

[1] Divisão de Endocrinologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil
[2] Departamento de Oncologia, Hospital Federal Cardoso Fontes, Rio de Janeiro, RJ, Brasil
[3] Programa de Engenharia Nuclear, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil
[4] Divisão de Reumatologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil

ABSTRACT

Objective: To evaluate changes in bone density and architecture in postmenopausal women with breast cancer (BC) and use of aromatase inhibitor (AI). **Subjects and methods:** Thirty-four postmenopausal women with BC, without bone metastasis, renal function impairment and who were not receiving bone-active drugs were selected from a population of 523 outpatients treated for BC. According to the presence of hormonal receptors, HER2 and Ki67, seventeen had positive hormonal receptors and received anastrozole (AI group), and seventeen were triple-negative receptors (non-AI group), previously treated with chemotherapy. Areal bone mineral density (aBMD) and vertebral fracture assessment (VFA) analyses were performed by DXA; vBMD and bone microarchitecture were evaluated by HR-pQCT. Fracture risk was estimated using the FRAX tool. **Results:** No patient referred previous low-impact fracture, and VFA detected one moderate vertebral fracture in a non-AI patient. AI patients showed lower aBMD and BMD T-scores at the hip and 33% radius and a higher proportion of osteoporosis diagnosis on DXA (47%) vs non-AI (17.6%). AI group had significantly lower values for vBMD at the entire, cortical and trabecular bone compartments, cortical and trabecular thickness and BV/TV. They also had a higher risk for major fractures and for hip fractures estimated by FRAX. Several HR-pQCT parameters evaluated at distal radius and distal tibia were significantly associated with fracture risk. **Conclusion:** AI is associated with alterations in bone density and microarchitecture of both the cortical and trabecular compartments. These findings explain the overall increase in fracture risk in this specific population. Arch Endocrinol Metab. 2021;65(4):505-11

Keywords

Breast cancer; osteoporosis; aromatase inhibitors (AI); bone mineral density (BMD); high resolution peripheral quantitative computed tomography (HR-pQCT)

Correspondence to:
Miguel Madeira/Felipe Peres Oliveira
Divisão de Endocrinologia,
Universidade Federal do Rio de Janeiro
Rua Professor Rodolpho Paulo Rocco, 255, 9º andar
21941-913 – Rio de Janeiro, RJ, Brasil
migmadeira@gmail.com / felipemb86@yahoo.com.br

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INTRODUCTION

Breast cancer (BC) is the most prevalent cancer type for women. Approximately 1.67 million new cases of BC were diagnosed in 2012 worldwide, representing 25% of all cancers in women. For Brazil, in 2018, 59,700 new cases of BC were expected (1). In 2019, the expectation is for 268,600 new cases and 41,760 deaths caused by this disease in the USA (2).

The presence of hormone receptors expressed by the tumor cells guides treatment. Approximately 70% of patients with BC have tumors with positive hormone receptors (R+), and those with potentially

curable disease benefit from adjuvant hormone therapy (3).

In postmenopausal (PM) women, estrogen synthesis depends mostly on the conversion of adrenal precursors by the enzyme aromatase, which is present in extragonadal sites, mainly adipose tissue (4). After menopause, a negative imbalance in bone remodeling is expected, with accelerated bone loss, especially in the first 15-20 years. Approximately 52% to 66% of this loss occurs due to estrogen deficiency, and the rest stems from aging (5). Aromatase inhibitors (AIs) block aromatase activity, making circulating levels of PM estrogen virtually undetectable. Several studies have shown the oncological benefit of AI treatment in PM women with (R+BC). Estrogenic activity suppression increases the bone loss rate to approximately 2.6% per year and favors fragility fractures (4,6,7).

In the USA, the economic impact of osteoporosis on the health care system is estimated to reach $ 25.3 billion per year by 2025 (8). In Brazil, there are currently 121,000 hip fractures per year, and projections are that numbers will rise to 140,000 in 2020 with enormous costs (about R$ 1.2 billion per year) (9). The only way of changing this picture is identify people at great risk and start antiosteoporosis treatment. The clinical evaluation of bone health is based on measures of areal bone mineral density (BMD) using dual X-ray absorptiometry (DXA) (10). A history of previous low-impact fractures or detection of non-clinical vertebral fractures on X-rays or during DXA exams (vertebral fracture assessment – VFA) also confirm osteoporosis. The Fracture Risk Assessment Tool (FRAX) is recommended by the World Health Organization to estimate the 10-year risk for hip and major fractures, and it was normalized in 2013 for the Brazilian population (9-11).

However, a considerable proportion of PM women and elderly men develop fragility fractures despite not having an osteoporosis diagnosis by DXA (12,13). In fact, BMD accounts for only 70%-75% of the variation in bone strength, while other factors (macro and microarchitecture, tissue composition and microcrack accumulation) correspond to the remainder of this variation. Within this context, the concept of bone quality has gained importance. Both bone compartments (cortical or trabecular) contribute to bone resistance, and they are differentially affected by age, gender, comorbidities and treatments (14,15). Three-dimensional high-resolution peripheral quantitative computed tomography (HR-pQCT) allows for evaluations of volumetric BMD and microstructure at the trabecular and cortical compartments, separately. This allows for a better understanding of alterations in bone geometry and strength associated with increased fracture risk (16).

This study aimed to evaluate the impact of AI on bone health of PM women with BC, based on the detection of previous fractures, estimation of fracture risk using FRAX, bone density and microarchitecture evaluated by DXA and HR-pQCT.

SUBJECTS AND METHODS

Subjects

This was a single-center, cross-sectional and observational study of PM women with BC admitted to Cardoso Fontes Federal Hospital (Rio de Janeiro, Brazil) (Ethics committee approval number: 64781417.0.0000.8066; Ethics committee's feedback number: 2.015.685). The BC diagnoses were based on histopathology. Specifically, immunohistochemistry evaluations were utilized to determine hormonal receptor and HER2 expression as well as Ki67 status in BC cells. Patients younger than 75 years on the second to fifth year of adjuvant AI therapy were eligible to participate in this research protocol. Age-matched patients with BC considered negative for hormonal receptors comprised the non-AI group. Clinical data were collected from medical records, and patients were interviewed about previous low-impact fractures. Every patient submitted to chemotherapy had discontinued this treatment for at least 2 years prior to entrance into this study. The exclusion criteria were as follows: weight above 120 kg (limitations of the densitometer), metastatic disease, other preexisting bone diseases (such as Paget's disease, hyperparathyroidism and hypoparathyroidism), renal failure, prednisone use ≥5 mg for 3 months or more) and use of anti-osteoporosis medications (e.g., bisphosphonates, denosumab and teriparatide).

The protocol was approved by the Research Ethics Committee of the hospital, and all patients received and signed the informed consent form before participating in the protocol, which was in accordance with the Second Declaration of Helsinki.

FRAX

The FRAX tool, adjusted for the Brazilian population, was employed to estimate a 10-year probability of hip and major fractures (9). All participants were considered to have secondary causes of osteoporosis.

Areal bone density and VFA

A Prodigy densitometer (GE Lunar Prodigy Advance, GE Healthcare, Madison, WI, USA) was used for DXA assessment of areal BMD at the lumbar spine, femoral neck, total femur and 33% radius, and the results were expressed as absolute values (g/cm^2) and standard deviations (SDs) from the expected BMD for young women (T-score). According to the ISCD criteria (10), patients were identified as having low bone density (previously referred to as osteopenia) or osteoporosis when the lowest BMD T-score was between < -1 and > -2.5 SD or ≤ -2.5 SD, respectively. Patients with a BMD Z-score ≤ -2 SD at any site were considered as having a lower than expected BMD for their age. The variability coefficients of the BMD values were estimated at 1.5% at the lumbar spine and 2.3% at the hip. VFA was also performed during the DXA examinations. The same accredited technician analyzed all images.

HR-pQCT

Volumetric BMD (vBMD) and bone microarchitecture were measured on the appropriately immobilized non-dominant distal forearm and tibia using a 3D HR-pQCT system (Xtreme CT, SCANCO Medical AG, Brüttisellen, Switzerland). This system employs a 2D detector combined with a 0.08-mm point-focus X-ray tube, which enables the acquisition of several CT sections with an 82-µm nominal resolution. A total of 110 sections were obtained at each site, generating a 9-mm 3D representation in the axial direction. The radiation dose was similar to that used in standard DXA procedures (less than 3 µSv per measurement). The attenuation data were transformed to equivalent hydroxyapatite (HA) densities. Additional details of image acquisition and analysis have been described previously (17). The variables included in the analysis were as follows: volumetric BMD (g HA/cm^3) in the trabecular (Dtrab), cortical (Dcomp) or total (Dtotal) region; cortical thickness (CTh, mm); fraction of trabecular bone volume to tissue volume (BV/TV); trabecular thickness (TbTh, mm); trabecular number (TbN, mm^{-1}); trabecular separation (TbSp, mm); and standard deviation of the TbSp (TbSp 1/N SD, mm), which reflects the heterogeneity of the trabecular network. TbTh and TbSp were calculated based on the TbN and BV/TV [TbTh = BV/TV/TbN, and TbSp (1-BV/TV)/TbN]. CTh was calculated by dividing the cortical volume by the external bone surface area. The variability of density-based measurements was less than 1% and between 3% and 5% for the bone structural parameters (18,19).

Statistics

The statistical analyses were performed using SPSS version 20.0 for MacOS (SPSS Inc., Chicago, IL, USA). In the descriptive analysis, the categorical variables were expressed through their percentages and frequencies. Numerical variables with a normal distribution were expressed as the mean ± standard deviation, while the variables with asymmetric distributions were expressed with medians (minimum - maximum). The Kolmogorov-Smirnov test was performed to evaluate the distribution pattern of the numerical variables. The Student's T-test or the Mann-Whitney U-test were performed to compare numerical variables between the two groups, as appropriate. The chi-squared or Fisher's exact tests were applied to compare categorical variables, as appropriate. Correlations between the numerical variables were analyzed using the Spearman correlation test. Bi-caudate tests were used in all analyzes. The limit of statistical significance was 5%.

RESULTS

A total of 34 patients participated in the study according to the criteria mentioned in Figure 1. Seventeen patients were assigned to the AI group and 17 patients to the non-AI group. The AI patients received letrozole or anastrozole for 2-5 years (mean 3.11 ± 1.00 years), six of them used tamoxifen and 9 had been on chemotherapy prior to the AI treatment. All receptor negative patients received chemotherapy. No difference was found between the AI and non-AI groups concerning age at the study (62.00 ± 5.80 *vs.* 57.05 ± 8.96 years, p = 0.066), age at menopause (47.94 ± 6.24 *vs.* 46.35 ± 4.74 years, p = 0.410), time elapsed since menopause (13.47 ±

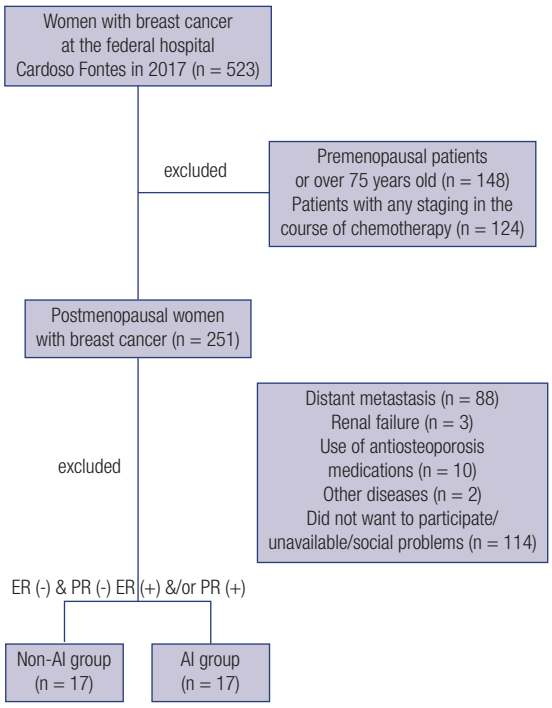

Figure 1. Flowchart of study subjects. A total of 251 postmenopausal women with breast cancer were pre-selected and 34 included. ER: estrogen receptor; PR: progesterone receptor; AI: aromatase inhibitor.

8.24 *vs.* 10.76 ± 9.79 years, p = 0.390), the number of patients referring regular physical exercise (10 *vs.* 8), type 2 diabetes mellitus (6 *vs.* 5), or ethnicity. The AI patients had lower body mass indexes (26.55 ± 3.22 *vs.* 30.88 ± 7.10 kg/m^2, p = 0.034).

There was no history of previous low-impact fracture in either group and VFA detected only one morphometric fracture in a non-AI patient (moderate fracture of T6). However, the FRAX-Brazil tool estimated a higher risk for major (AI 3.47% ± 1.57 *vs.* non-AI 2.50% ± 0.76, p = 0.029) and hip fractures (AI 0.50% [0-4.6] *vs.* non-AI 0.20% [0-1.9], p = 0.010).

Bone densitometry revealed decreased BMD and T-scores in the hips and 33% radius (Table 1). Furthermore, the distribution of patients classified as having normal BMD (2 AI *vs.* 9 non-AI), low BMD (7 AI *vs.* 5 non-AI) and osteoporosis (8 AI *vs.* 3 non-AI) was significantly different between the groups (p = 0.029). Additionally, 5 AI patients and only 1 non-AI patient had lower than expected BMD for their age. The HR-pQCT confirmed alterations in bone density and differences in the bone microarchitecture between the groups (Table 1).

The only microstructural parameter significantly associated with age was Dcomp, at the distal radius (r -0.379, p = 0.027) and at the distal tibia (r -0.454, p = 0.007). BMI was positively associated with Ct.Th at the radius (r 0.352, p = 0.041) and tibia (r 0.367, p = 0.033) as well as with Dcomp (r 0.367, p = 0.033) and TbN (r 0.364, p = 0.034), both at the distal tibia.

Fracture risk estimated using the FRAX tool was also negatively influenced by lumbar spine BMD (r -0.456, p = 0.008 for major fractures and r -0.455, p = 0.008 for hip fractures) and bone microstructure, including trabecular and cortical indexes, as shown in Table 2.

DISCUSSION

This study demonstrates that AIs used in BC patients is associated with higher risk of fragility fractures (evaluated by FRAX) and decreased bone density and quality (assessed by DXA and HR-pQCT) with negative effect on both trabecular and cortical bones.

Several studies have evaluated the effect of AIs on areal bone density as well as its therapeutic possibilities (20). In the ATAC study, for example, routine BMD monitoring showed that the highest rate of bone loss occurs in the first two years of AI use. There was a decrease in lumbar spine and hip BMD levels in the AI patients (6.08% and 7.24%, respectively) in contrast with those on tamoxifen, who showed a BMD gain of 2.88% and 0.74%, respectively. AI discontinuation led to a BMD increase at the lumbar spine and no further hip loss (21).

A better understanding of bone properties can be obtained by the histomorphometry analysis of bone biopsy, which is an invasive and expensive method. QCT and HR-pQCT for bone study indirectly assess these parameters and help to clarify the changes associated with bone fragility.

There is a growing number of clinical trials using QCT and HR-pQCT for evaluation of bone quality; however, only a few have evaluated BC patients. Lee and cols.(22) utilized QCT in the lumbar spine and femur to study the influence of AIs on bone. Like our DXA data, they did not find differences in lumbar spine BMD, but vBMD was decreased in the femoral neck and total femur in their AI patients. The cortical bone compartment was especially affected in their AI patients, which is in accordance with our

Table 1. Bone densitometry and high-resolution peripheral quantitative computed tomography data of patients with breast cancer receiving aromatase inhibitors (AI) or not (non-AI)

	AI (n = 17)	Non-AI (n = 17)	p-value
DXA			
LS BMD (g/cm^2)	1.030 ± 0.159	1.121 ± 0.165	0.127
FN BMD (g/cm^2)	0.901 ± 0.081	0.992 ± 0.123	**0.018**
TF BMD (g/cm^2)	0.910 ± 0.098	1.013 ± 0.127	**0.014**
33%R BMD (g/cm^2)	0.583 ± 0.073	0.694 ± 0.064	**<0.001**
HR-pQCT			
radius			
D100 (mgHA/cm^3)	272.01 ± 41.23	341.58 ± 61.51	**0.001**
D comp (mgHA/cm^3)	836.80 ± 52.31	890.70 ± 57.60	**0.009**
Ct.Th (mm)	0.64 ± 0.12	0.80 ± 0.16	**0.003**
D trab (mgHA/cm^3)	124.01 ± 29.36	163.35 ± 37.88	**0.002**
BV/TV	0.10 ± 0.02	0.14 ± 0.03	**0.003**
Tb.N (mm^{-1})	1.75 ± 0.32	1.95 ± 0.26	0.055
Tb.Th (mm)	0.06 ± 0.01	0.07 ± 0.01	**0.007**
Tb.Sp (mm)	0.54 ± 0.12	0.45 ± 0.08	**0.032**
Tb.1/N.SD (mm)	0.26 ± 0.11	0.20 ± 0.05	0.098
tibia			
D100 (mgHA/cm^3)	261.50 ± 44.10	296.44 ± 66.10	0.088
D comp (mgHA/cm^3)	833.90 ± 59.40	884.47 ± 61.21	**0.024**
Ct.Th (mm)	1.07 ± 0.19	1.17 ± 0.28	0.233
D trab (mgHA/cm^3)	130.74 ± 30,56	153.31 ± 38.03	0.074
BV/TV	0.11 ± 0.03	0.13 ± 0.03	0.073
Tb.N (mm^{-1})	1.55 ± 0.31	1.70 ± 0.30	0.158
Tb.Th (mm)	0.07 ± 0.01	0.07 ± 0.01	0.327
Tb.Sp (mm)	0.61 ± 0.17	0.53 ± 0.09	0.111
Tb.1/N.SD (mm)	0.35 ± 0.20	0.25 ± 0.06	0.088

LS: lumbar spine; FN: femoral neck; TF: total femur; 33%R: radius 33%; D100: vBMD of entire bone; D comp: cortical vBMD; Ct. Th: cortical thickness; D trab: trabecular vBMD; BV/TV: bone volume/total volume ratio; Tb. N: trabecular number; Tb. Th: trabecular thickness; Tb.Sp: trabecular separation; Tb.1/N.SD: inhomogeneity of trabecular network.

Table 2. Correlations between microstructural parameters and fracture risk estimated by FRAX

	FRAX Major fracture		FRAX Hip fracture	
	r	p-value	r	p-value
Radius				
D100	-0.499	0.003	-0.487	0.004
D comp	-0.573	<0.001	-0.551	0.001
Ct.Th	-0.587	<0.001	-0.561	0.001
D trab	-0.388	0.023	-0.387	0.024
BV/TV	-0.390	0.023	-0.390	0.023
Tibia				
D100	-0.631	<0.001	-0.365	<0.001
D comp	-0.709	<0.001	-0.698	<0.001
Ct.Th	-0.544	0.001	-0.540	0.001
D trab	-0.513	0.002	-0.524	0.001
BV/TV	-0.517	0.002	-0.527	0.001
Tb.N	-0.456	0.007	-0.426	0.012
Tb.1/N.SD	0.477	0.004	0.468	0.005

findings of deleterious effects on the cortical bone. The authors also reported that bone loss in their AI patients was negatively related to age and time on AI treatment and was positively associated with BMI. The authors concluded that AI treatment was associated with deterioration of femoral cortical BMD and geometry, which could contribute to site-specific decreased bone strength and increased incidence of hip fractures (22).

Szabo and cols. (23) utilized peripheral QCT (pQCT) to compare BC patients on AI treatment and healthy PM women. Their AI patients demonstrated significantly lower total vBMD values (4% at radius and at tibia) and lower cortical densities (20% at radius and 38% at tibia). We found similar data, such as significant decreases in total bone, cortical and trabecular vBMDs, but the HR-pQCT could also detect decreased trabecular and cortical thickness as well as reduced trabecular BV/TV.

Only one study utilized HR-pQCT and DXA in patients with BC, but in patients receiving AI drug (exemestane) for 2 years (24). There was a significant decline in aBMD (DXA) in the lumbar spine, femoral neck and total femur, as well as in the total, cortical and trabecular vBMD and cortical thickness (radius and tibia) and BV/TV in the distal radius. We also found similar alterations in both compartments but mainly in the cortical bone in patients receiving anastrozole or letrozole.

There are several tools for evaluating fracture risk related to osteoporosis – FRAX being the most utilized. It contemplates several risk factors, including secondary causes of osteoporosis, such as the use of drugs interfering with bone health, although not specifically AIs (11). Mariotti and cols. (25) showed that the combination of FRAX, trabecular bone score and BMD maximized the identification of BC patients with elevated fracture risk. Cheung and cols. (24) evaluated fracture risk based on the FRAX tool in patients receiving exemestane but could not conclude that a decline occurred in their 2-year study period.

In this study, the group receiving AI had a higher FRAX fracture risk for major and hip fractures. We searched for previous fractures in the patient histories as well as for non-clinical vertebral fractures by VFA, and we believe that the absence of fractures might be due to their short times on AIs and the small sample size (type 2 error). The lower BMI in the AI patients may have contributed to alterations in bone microstructure at the distal tibia, which is a weight bearing bone. The most important finding was the significant correlation of fracture risk with bone microarchitecture, both in the trabecular and cortical compartments, which was undoubtedly deranged in the AI patients.

Our study has limitations due to its cross-sectional design and the small sample size. However, we detected a clear deterioration in bone density and microarchitecture in both cortical and trabecular bone using HR-pQCT, which might explain the bone fragility and increased fracture risk in the BC patients receiving AIs. An important information regarding the two subgroups is that the patients in the control group (triple negative) had higher body weight, which may give this group a protective factor against bone loss. However, also in this group, all patients received chemotherapy, which can lead to loss of bone mass. Another relevant data is the use of the subtypes of AIs. Some studies have suggested a less significant effect of exemestane on bone loss, due to its androgenic structure, compared to letrozole, while other clinical studies comparing different AIs did not reveal any significant difference (26). In our study, due to standardized medication in the hospital, anastrozole was basically used as a drug in the adjuvant treatment.

We are facing a public health problem that affects many people worldwide: BC and osteoporosis. Our study reinforces the importance of assessing bone health early in women diagnosed with BC, with PM status who will use adjuvant therapy with AIs. Early diagnosis can thus prevent bone events, financial cost in their treatments and worsening quality of life.

The use of HR-pQCT adds valuable information to the understanding of women's bone health and fracture risk. Future studies with a larger population of BC patients receiving AIs may deepen the understanding of how bone is impacted by this treatment modality.

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Cyclic ACTH-secreting thymic carcinoid: a case report and review of the literature

[1] Unidade de Medicina Interna e Serviço de Endocrinologia, Faculdade de Medicina e Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil
[2] Unidade de Neuroendocrinologia, Instituto Estadual do Cérebro Paulo Niemeyer, Rio de Janeiro, RJ, Brasil
[3] Laboratório de Neuropatologia e Genética Molecular, Instituto Estadual do Cérebro Paulo Niemeyer, Rio de Janeiro, RJ, Brasil
[4] Divisão de Medicina Nuclear, Centro de Imagem Copa D'Or e Hospital Copa Star, Rio de Janeiro, RJ, Brasil
[5] Unidade de Cirurgia Torácica, Rede D'Or São Paulo e Hospital Copa Star, Rio de Janeiro, RJ, Brasil
[6] Divisão de Cirurgia Torácica, Instituto do Câncer do Estado de São Paulo, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil
[7] Divisão de Oncologia, Instituto Oncoclínicas, Pesquisa e Educação, Rio de Janeiro, RJ, Brasil
[8] Divisão de Patologia, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brasil
[9] Unidade de Cirurgia Torácica, Rede D'Or Rio de Janeiro e Hospital Copa Star, Rio de Janeiro, RJ, Brasil
[10] Unidade de Cirurgia Torácica, Pontifícia Universidade Católica do Rio de Janeiro, Rio de Janeiro, RJ, Brasil

Correspondence to:
Mônica R. Gadelha
Rua Professor Rodolpho Paulo Rocco, 255, 9° andar, Setor 9F, Centro de Pesquisa em Neuroendocrinologia, Hospital Universitário Clementino Fraga Filho
21941-913 – Rio de Janeiro, RJ, Brasil
mgadelha@hucff.ufrj.br

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Elisa B. Lamback[1,2,3]
https://orcid.org/0000-0002-6026-4329

Sérgio Altino de Almeida[4]
https://orcid.org/0000-0001-5667-5582

Ricardo Terra[5,6]
https://orcid.org/0000-0002-5084-6704

Carlos Gil Ferreira[7]
https://orcid.org/0000-0002-7228-7018

Vera Luiza Capelozzi[8]
https://orcid.org/0000-0001-9732-5853

Rui Haddad[9,10]
https://orcid.org/0000-0002-1288-3539

Mônica R. Gadelha[1,2,3]
https://orcid.org/0000-0002-9250-3558

SUMMARY

Cyclic Cushing's syndrome (CS) due to thymic carcinoid is a rare disorder. We report a case of cyclic CS due to ectopic adrenocorticotropic hormone (ACTH)-secreting atypical thymic carcinoid tumor and reviewed similar cases published in the literature. Our patient had hypercortisolemia lasting approximately one month, followed by normal cortisol secretion, with relapse one year later. Histopathology revealed an atypical ACTH-positive thymic carcinoid. Ectopic CS can be derived from atypical thymic carcinoids, which can be aggressive tumors with early relapse, suggesting that this type of tumor probably needs aggressive treatment. Arch Endocrinol Metab. 2021;65(4):512-6

INTRODUCTION

Cushing's syndrome (CS) is a rare disorder characterized by inappropriately elevated secretion of cortisol (1). The syndrome has an estimated annual incidence of 0.2 to 5 per million persons (2). Most cases are caused by the overproduction of adrenocorticotrophic hormone (ACTH) by a pituitary corticotropinoma (2). Approximately 10% of CS cases result from the ectopic secretion of ACTH and are mainly derived from the foregut (larynx, thymus, lungs, stomach, duodenum and pancreas) (3,4). Approximately 100 cases of CS arising from thymic neuroendocrine tumor (NET) have been described to date (2,5). In rare cases, these thymic NET can be associated with cyclic CS (1).

We report a case of cyclic CS caused by a thymic NET and review similar cases published in the literature.

CASE REPORT

A 32-year-old Caucasian man presented with sudden and unexplained weight gain (nine kilograms in a month), decreased libido, arterial hypertension and acne at 30 years of age, which resolved spontaneously. One year later, he developed the same rapid-onset signs and symptoms, along with severe anxiety and panic attacks that required

clonazepam treatment. At this time, the patient was referred to our institution, and we noted in retrospect that he had been investigated for decreased libido at the age of 29 years, being diagnosed and treated for central hypogonadism [total testosterone: 171 ng/dL [reference value (RV): 220-819; FSH: 5.2 mUI/mL (RV: 1.5-12.4); LH: 3.5 mUI/mL (RV: 1.7-8.6); prolactin: 7.3 ng/mL (RV: 2.0-15.2)] with no further investigation. Physical examination revealed a typical cushingoid appearance, with facial plethora, moon face, central obesity, acne on his thighs, arterial hypertension and mild tachycardia. Cushing's syndrome was investigated, and biochemical assessment was compatible with ACTH-dependent CS: urinary free cortisol (UFC): 8077.5 mcg/24 hours (RV: 21-111); 11 p.m. salivary cortisol: 8.3 mcg/dL (RV: <0.2); ACTH: 178 and 264 pg/mL (RV: < 46); 8 a.m. basal cortisol: > 60 and 119.1 mcg/dL. The dehydroepiandrosterone sulfate level was 1947 mcg/dL (RV: 99-449), and the potassium level was normal at 4.6 mEq/L (RV: 3.5-5.3). He also showed central hypogonadism: total testosterone: 199 ng/dL (RV: 220-819); FSH: 2.1 mUI/mL (RV: 1.5-12.4); LH: 1.9 mUI/mL (RV: 1.7-8.6).

No pituitary lesion was observed on magnetic resonance imaging (MRI). Bilateral inferior petrosal sinus sampling (BIPSS) demonstrated an ectopic origin for the hypercortisolemia (the ratio between central and peripheral ACTH was 1.2 at baseline and 2.3 after 10 mcg of endovenous desmopressin). BIPSS was performed when the patient was likely exciting the active phase and entering normocortisolemia, as suggested by 8 a.m. basal cortisol value of 14.6 mcg/dL and still increased ACTH levels of 54.9 pg/mL measured six days after the biochemical confirmation of ACTH-dependent CS as stated above. UFC was not measured on the day of BIPSS. Even done at this stage, BIPSS was compatible with an ectopic origin (the gradient between central and peripheral ACTH was <2 at baseline and <3 after desmopressin). Computed tomography (CT) of the chest revealed an anterior mediastinal mass suggesting a thymic lesion (Figure 1). Octreotide receptor scintigraphy (octreoscan) demonstrated increased uptake of the tracer in the anterior mediastinum. The patient showed no evidence of carcinoid syndrome. He had normocalcemia, a normal chromogranin A level of 2.4 nmol/L (RV: < 3.0) and a negative family history of multiple endocrine neoplasia type 1 (MEN1).

Similar to the previous episode, the signs and symptoms of CS resolved spontaneously after 30 to 40 days, with significant weight loss of 10.5 kg in this period and the 8 a.m. cortisol levels decreasing to 10.3 mcg/dL, suggesting cyclic CS. He showed no clinical or biochemical evidence of adrenal insufficiency. The central hypogonadism at the age of 29 years was attributed to inhibition of the hypothalamus-pituitary-gonadal axis due to the hypercortisolemia that was likely already present at this age.

Robotic thymectomy was performed with a surgical description of complete tumor removal. The surgical specimen showed a tumor of 1.5 cm and several carcinoid tumorlets (<1 cm) in the surrounding abundant fat (Figure 2). Microscopically, the tumor was comprised of uniform cells with nested, trabecular, and rosette-like growth patterns. The polygonal tumor cells had moderate eosinophilic granular cytoplasm, round to oval nuclei, "salt and pepper" chromatin and inconspicuous nucleoli (Figure 3A). Large zones of necrosis were present, and the mitotic count varied from 4 to 6 mitoses/mm^2. Immunohistochemistry of the tumor samples was positive for CD56, chromogranin A, synaptophysin, and ACTH, and the Ki-67 index greater than 35% (Figures 3B-D).

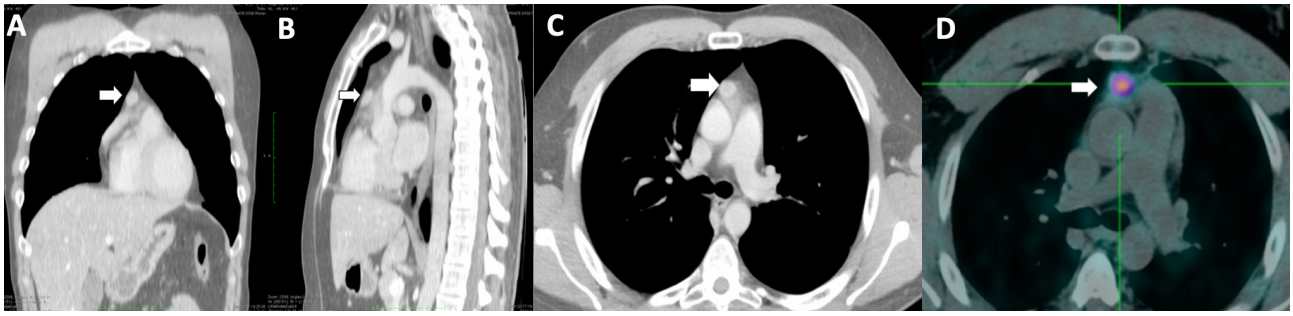

Figure 1. Chest computed tomography scan in the coronal (**A**), sagittal (**B**) and axial (**C**) views showing a 2.0×1.1×1.4 cm tumor in the anterior mediastinum (arrows) and ^{68}Ga-DOTATOC-PET CT scan in the axial view (D) exhibiting focal radiotracer uptake in the anterior mediastinum (SUVmax = 6.7/15×11 mm).

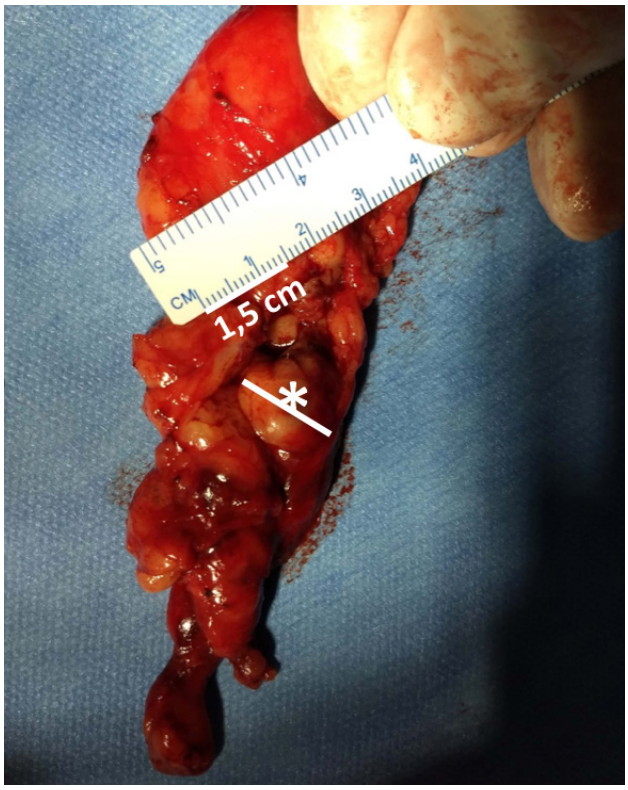

Figure 2. Surgical specimen with the tumor (*) measuring 1.5 cm with the thymic remnant showing several carcinoid tumorlets in the surrounding abundant fat.

On postoperative assessment, the patient required glucocorticoid coverage for a short period (1.5 months). The ACTH level was normalized to 43.6 pg/mL. After three months of surgery, he had normal free urinary cortisol and 11 p.m. salivary cortisol but nonsuppression in an overnight dexamethasone suppression test [serum cortisol: 2.6 mcg/dL (RV: <1.8)]. The chromogranin A levels increased to 3.3 nmol/L (RV: <3.0), and ACTH increased to 71.6 pg/mL four months after surgery. At this time, the ^{68}Ga-DOTATOC-PET CT scans demonstrated focal uptake in the anterior mediastinum (Figure 1), with mild radiotracer uptake in the same location as ^{18}F FDG-PET CT. Although the patient had undergone complete surgical resection and remained asymptomatic, an increase in ACTH, chromogranin A and focal uptake in PET CT scans may indicate early relapse of an aggressive tumor. He remains on hypertensive medications, and has normal glycemic levels and a normal bone mineral density.

Considering the above findings, the patient was diagnosed with cyclic CS due to ACTH-secreting atypical thymic carcinoid based on the 2015 World Health Organization classification for neuroendocrine neoplasms.

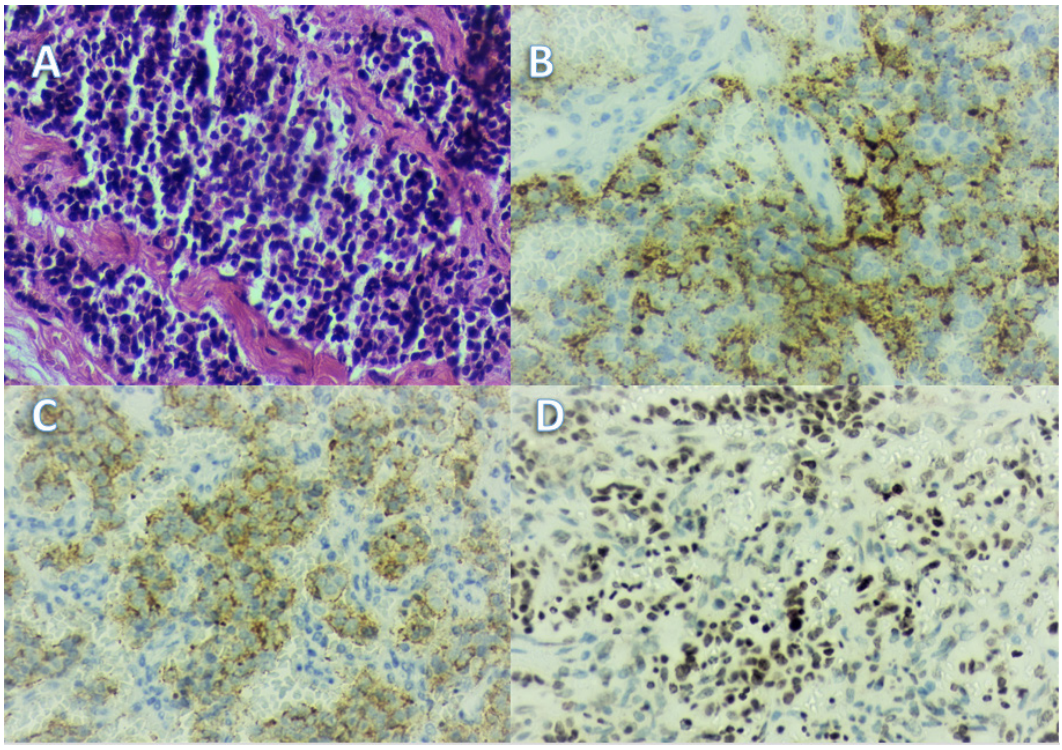

Figure 3. Histopathology of atypical thymic carcinoid. (**A**) Hematoxylin-eosin staining, ×200, showing a solid and trabecular growth pattern among a delicate vascularized fibroconjunctival stroma; (**B**) Immunohistochemistry, ×200, showing granular membranous ACTH staining; (**C**) Immunohistochemistry, ×200, showing strong membranous chromogranin A staining; (**D**) Immunohistochemistry, ×200, showing a high Ki67 proliferation index.

DISCUSSION

We report a case of a male patient who presented with rapid-onset symptoms suggestive of hypercortisolemia that occurred during cycles that lasted approximately one month and relapsed every year for two years before the diagnosis of ectopic CS due to atypical thymic carcinoid. Approximately 100 cases describing CS resulting from ACTH secretion from thymic tumors have been reported in the literature, with fewer than 10 cases of cyclic CS due to ectopic ACTH secretion from a thymic NET (1,5-11).

Thymic NET are rare tumors with an annual incidence of 0.07 to 0.18 per million persons, with higher rates in Caucasians and male individuals (12). However, thymic ACTH-producing NET exhibit no gender preference and are usually diagnosed in early adulthood (21-35 years of age) (13). Functionally active tumors are seen in one-third to half of cases (13). Thymic carcinoids are associated with MEN 1 in up to 25% of cases (13).

In ectopic ACTH secretion, the ACTH plasma levels are usually very high, as observed in our case (1). Because most NET express somatostatin receptors, octreotide receptor scintigraphy (octreoscan) or PET CT with ^{68}Gallium-labeled somatostatin analogs can be used to evaluate these patients, including in cases of ectopic ACTH-secreting tumors (14-16).

NET produce biogenic amines such as serotonin which may lead to carcinoid syndrome. NET originate from one of three portions of the primitive gut: foregut, midgut and hindgut. Thymic NET are foregut tumors, which have lower serotonin metabolism than midgut tumors, reflecting a low frequency of carcinoid syndrome in these patients, as observed in our case (1).

Patients with CS (from different etiologies) have episodic cortisol secretion (17). However, in a small subset of CS patients, highly variable levels of glucocorticoid secretion can occur. Episodes of hypercortisolemia interspaced with periods of normocortisolemia (or adrenal insufficiency) are known as cyclic CS (1). Adrenal insufficiency can also be observed, but was not seen in our case, probably because of the short cycle length. In cyclic CS, rhythmic fluctuations in ACTH secretion occur and result in more or less predictable cyclic variation in adrenal steroid production (18). The cycle length usually lasts one month and the intercyclic phase is prolonged in ectopic secretion of ACTH, as seen in our case (1). Meinardi and cols. (1) reviewed 65 cases of cyclic CS cases. Most of the cases were due to Cushing's disease (CD), followed by the ectopic secretion of ACTH in 26% of cases. Considering that approximately 10% of CS cases are due to ectopic ACTH secretion, cyclic CS seems to occur more frequently in patients with ectopic ACTH secretion (1).

Based on literature reports of thymic NET causing cyclic CS published to date, the epidemiology is similar to thymic NET causing CS. The diagnosis usually occurs in early adulthood (20-43 years old), with no gender predilection (1,5-9,11,13). Nevertheless, one case was described in a 7-year-old child (10). CS cycles may last days or months or are seasonal, as described by Trott and cols. (5). Additionally, symptoms of hypercortisolemia may not be typical because of the cyclic nature of the ACTH production. Furthermore, hypokalemia and osteoporosis, which are more frequent in ectopic CS, are not always present in cyclic CS and was not observed in our case (3). Nonetheless, cortisol-induced comorbidities should be monitored regularly. Cyclic CS also seems to cause more psychiatric disturbances than noncyclic CS, as observed in our case.

Cyclic CS is difficult to diagnose, requiring clinical suspicion and repeated testing. Dynamic testing is often inconclusive due to unsustained hypercortisolemia. The mechanism underlying cyclic CS remains unclear. A proposed explanation would be episodes of spontaneous tumor hemorrhage or cyclic growth and apoptosis of ACTH-secreting tumor cells (19,20).

The 2015 WHO classification of thymic neuroendocrine tumors is generic but accurate, and the lesions are classified into three grades according to the mitotic count and presence of necrosis (21). Low-grade (typical carcinoid) lesions have 2 or fewer mitotic counts and no necrosis, intermediate-grade (atypical carcinoid) lesions have 2-10 mitotic counts and foci of necrosis, and high-grade (large cell neuroendocrine carcinoma and small cell carcinoma) lesions have more than 10 mitotic counts and the presence of necrosis. The distinction between an atypical neuroendocrine tumor (AC) and a large cell neuroendocrine carcinoma (LCNEC) is difficult based solely on the 2015 WHO pathological classification. Other pathological classifications and clinicopathological correlations are required. Applying the Ki-67-based ENETS classification, which shows that the Ki-67 index of AC varies on average from 1% to 18.8% and that of LCNEC varies from 16% to 90%, our case would be considered LCNEC (22,23). However,

based on early relapse, mitotic count and high Ki-67 index, the tumor was classified as overlapping between AC and LCNEC and considered an aggressive atypical neuroendocrine tumor.

Surgical treatment, with complete tumor removal, is the treatment of choice. Despite aggressive treatment, thymic NET have a poor prognosis. The ten-year survival rate is 38%, with worse outcomes in patients with CS (24-26). The role of chemotherapy has not been well established because the low number of studied patients limits its assessment (1).

Despite the resolution of hypercortisolemia with the resection of thymic tumors in our case, the patient had an ACTH level in the upper limit of the normal range and a nonsuppression response to the overnight dexamethasone suppression test, which could represent an unfavorable prognosis.

In conclusion, cyclic CS represents a clinical challenge requiring clinical suspicion. Ectopic CS can be derived from atypical ACTH-producing thymic carcinoids, which can relapse early, even after complete surgical removal. This outcome shows that this type of aggressive disease likely requires aggressive treatment.

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Medical adherence in the time of social distancing: a brief report on the impact of the COVID-19 pandemic on adherence to treatment in patients with diabetes

Debora Wilke Franco[1,2]
https://orcid.org/0000-0001-8531-8445

Janine Alessi[3]
https://orcid.org/0000-0003-4311-3307

Alice Scalzilli Becker[2]
https://orcid.org/0000-0003-0781-590X

Bibiana Brino do Amaral[2]
https://orcid.org/0000-0001-5561-9378

Giovana Berger de Oliveira[2]
https://orcid.org/0000-0001-8071-9741

Beatriz D. Schaan[3,4,5]
https://orcid.org/0000-0002-2128-8387

Gabriela Heiden Telo[1,2,6]
https://orcid.org/0000-0001-9093-383X

[1] Programa de Pós-graduação em Medicina e Ciências da Saúde, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brasil
[2] Escola de Medicina, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brasil
[3] Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil
[4] Escola de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil
[5] Divisão de Endocrinologia, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brasil
[6] Departamento de Medicina Interna, Hospital São Lucas, Pontifícia Universidade Católica do Rio Grande, Porto Alegre, RS, Brasil

Correspondence to:
Debora Wilke Franco
Universidade Pontifícia Católica do Rio Grande do Sul
Av. Ipiranga, 6.681, prédio 12, 2º andar – 90619-900 - Porto Alegre, RS, Brasil
debora.franco@edu.pucrs.br

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ABSTRACT

We conducted a cross-sectional study to evaluate the impact of social distancing determined by the COVID-19 pandemic on treatment adherence using the Self-Care Inventory-revised in adults with diabetes mellitus. In type 1 diabetes, the adherence score was lower during than before social distancing. Arch Endocrinol Metab. 2021;65(4):517-21

Keywords

Medical adherence; COVID-19 pandemic; diabetes mellitus; adherence to treatment; social distancing

INTRODUCTION

Treatment adherence is the main factor in the management of hyperglycemia and, consequently, in the reduction of diabetes-related complications. Stressful events have a significant impact on treatment adherence in patients living with diabetes mellitus, and the COVID-19 pandemic provides further reason for concern about these patients, who are among those at highest risk (1-5). In recent months, Brazil has become one of the pandemic epicenters in the world, with approximately 2,100,000 confirmed cases so far (6). This scenario may directly impact diabetes care due to poor availability of medical appointments, increased difficulty in obtaining medications, and home isolation.

The intrinsic differences in type of diabetes may impact patients with type 1 and type 2 diabetes differently during the pandemic. While spending more time at home could facilitate proper adherence to treatment guidelines, maintaining healthy eating habits and exercising may be challenging during quarantine. Therefore, the present study aimed to evaluate adherence in a cohort of patients with type 1 and type 2 diabetes during social distancing.

METHODS

Study design and setting

We conducted a controlled cross-sectional study to evaluate aspects of treatment adherence in a cohort of patients with type 1 and type 2 diabetes during social distancing. We invited patients to participate in the study by phone calls, during which the informed consent form was read aloud and any questions from potential participants were answered or clarified.

Each patient's informed consent was documented through audio recording. We administered a specific questionnaire to evaluate treatment adherence 1 month after the publication of the national recommendation of social distancing for high-risk groups for COVID-19 in Brazil.

Participants

We selected patients under regular monitoring at the endocrinology outpatient clinic of a tertiary public hospital in southern Brazil and divided them into 2 groups: social distancing group and control group. The inclusion criteria were previous diagnosis of type 1 or type 2 diabetes, age ≥18 years, HbA1c measured 3 months prior to inclusion in the study, and updated contact information in the electronic database. We excluded patients with any physical or cognitive impairment that could limit questionnaire administration and those who were hospitalized at the time of recruitment. For the social distancing group, we also excluded patients who were not following other social distancing rules besides the current national recommendation for high-risk groups. For the control group, we selected patients from 2 previous cohorts designed to evaluate treatment adherence before the pandemic. The data were collected in 2014 for the type 1 diabetes control group and in 2016 for the type 2 diabetes control group. Patients in the social distancing and control groups were matched for age for the present study.

Study outcome

We used a validated Brazilian Portuguese version of the Self-Care Inventory-revised (SCI-R) (7-8) to evaluate treatment adherence before and during the pandemic. We asked participants to answer the 14 questions with frequency descriptors. A score of 1 to 5 is assigned to each item, and a final score ranging from 14 to 70 is then calculated. Higher scores indicate greater adherence to diabetes treatment.

Clinical variables

We collected demographic and clinical variables from medical records and confirmed the data during the study telephone evaluation.

Sample size

Considering an estimated 27% increase in the risk of non-adherence in situations of anxiety and depression and the prevalence of poor adherence described in the literature of 49.1% in type 1 diabetes and of 42.0% in type 2 diabetes, we calculated that 110 patients with type 1 diabetes and 150 patients with type 2 diabetes would be needed to perform an analysis with 80% power and alpha of 0.05 (9-11).

Statistical analysis

We presented the data as mean (SD), median and interquartile range (IQR), or percentages. We used unpaired *t* test for continuous variables and $\chi 2$ test for categorical variables. We compared the social distancing and control groups using the Mann-Whitney test for nonparametric data. We stratified the analyses according to type of diabetes. Because the time of evaluation differed between the social distancing group and the control groups, we included a variable called 'calendar year' in a linear regression model to adjust for possible differences related to improvements in diabetes care over time. Considering that the effect of time is expected to be positive on treatment-adherence parameters, the adjustments for calendar year are presented only when the adherence score has increased in the social distancing group, as this group represents the most recently assessed one for diabetes care. We analyzed the data in SPSS, version 20, and set the level of statistical significance at $p \leq 0.05$ for all analyses.

Ethical aspects

The study was approved by the institution's research ethics committee (number 4.029.368) and reported following the STROBE guidelines (12). The project is registered at the Brazilian platform for research involving human participants called Plataforma Brasil (https://plataformabrasil.saude.gov.br/login.jsf), number 30528620.1.0000.5327.

RESULTS

Characteristics of the participants

We included 260 participants in the study. Overall, mean age was 43.7 (SD 12.7) years; 45.5% were female and 97.3% were white (see Table 1). The social distancing and control groups were similar in terms of demographics and clinical variables. There was also no difference in treatment regimens between the groups.

Table 1. Demographics and clinical characteristics of study participants

TYPE 1 DIABETES	Total (N = 110)	Social distancing group (n = 55)	Control (n = 55)	P value
Age (years)	43.7 ± 12.7	43.4 ± 13.8	43.9 ± 11.7	0.81
Sex (% female)	45.5%	49.1%	45.5%	0.44
Race/ethnicity (% white)	97.3%	96.4%	98.2%	0.22
Age at diabetes diagnosis (years)	19.2 ± 11.4	18.4 ± 12.5	19.9 ± 10.2	0.47
Diabetes duration (years)	24.6 ± 11.4	24.9 ± 11.8	24.1 ± 11.0	0.70
HbA1c (%) (mmol/mol)	8.6 ± 1.6 71.0 ± 17.9	8.6 ± 1.5 71.0 ± 15.9	8.6 ± 1.8 71.0 ± 19.7	1.0
Diabetes complications				
Retinopathy	48.2%	49.1%	47.3%	0.83
Neuropathy	17.3%	23.6%	10.9%	0.08
BMI overweigh/obese (%)	46.3%	45.3%	47.3%	0.83
Hypertension (%)	21.8%	25.5%	18.2%	0.35
Cardiovascular disease (%)	11.8%	12.7%	10.9%	0.76
TYPE 2 DIABETES	**(N = 150)**	**(n = 75)**	**(n = 75)**	
Age (years)	61.3 ± 7.9	61.8 ± 8.7	60.7 ± 7.0	0.40
Sex (% female)	60.7%	63.5%	58.7%	0.38
Race/ethnicity (% white)	74.0%	78.4%	70.7%	0.09
Age at diabetes diagnosis (years)	43.6 ± 10.6	42.8 ± 9.7	44.2 ± 11.4	0.42
Diabetes duration (years)	17.6 ± 9.0	18.8 ± 9.5	16.5 ± 8.6	0.12
HbA1c (%) (mmol/mol)	8.8 ± 1.5 73.0 ± 16.4	8.9 ± 1.5 74.0 ± 16.4	8.6 ± 1.5 70.0 ± 16.4	0.36
Diabetes complications				
Retinopathy	46.0%	43.2%	48.0%	0.46
Neuropathy	34.0%	29.7%	38.7%	0.39
Nephropathy	46.7%	44.6%	49.3%	0.54
Insulin use (%)	84.7%	89.2%	80.0%	0.27
BMI overweigh/obese (%)	91.0%	91.3%	90.7%	0.94
Hypertension (%)	82.6%	82.4%	82.7%	0.89
Cardiovascular disease (%)	38.7%	41.9%	36.0%	0.55

Data are presented as mean ± SD or %. $\alpha \leq 0.05$ indicates significant difference. HbA1c: hemoglobin A1c; BMI: body mass index.

Outcomes

In type 1 diabetes, the median SCI-R score was lower in the social distancing group (48.0, IQR 41.0-52.0) than in the control group (52.0, IQR 46.0-54.0) ($p < 0.01$) (see Figure 1). In type 2 diabetes, the median SCI-R scores were similar in the social distancing (48.0, IQR 43.0-55.0) and control groups (47.0, IQR 44.0-51.0) ($p = 0.14$).

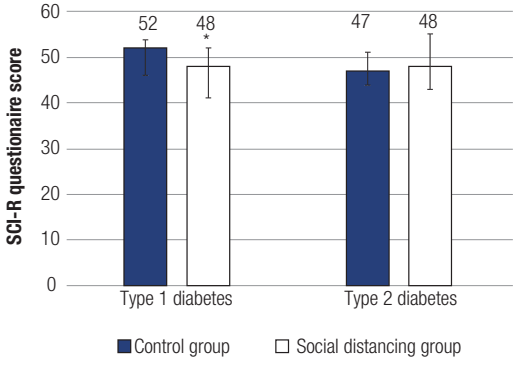

Figure 1. Adherence scores among patients with type 1 and type 2 diabetes before and during social distancing determined by COVID-19.

Data are presented as median and interquartile range. *: α level ≤ 0.05, indicating significant difference. The validated Brazilian version of the Self-Care Inventory-revised (SCI-R) was used for this evaluation. Scores range from 14 to 70. Higher scores indicate greater adherence to diabetes treatment.

DISCUSSION

This study found no difference in adherence scores among patients with type 2 diabetes before and during social distancing related to the COVID-19 pandemic. Although spending more time at home could facilitate

proper adherence to treatment guidelines, adherence scores worsened among patients with type 1 diabetes during social distancing.

This study has some limitations. As the study has a cross-sectional design, our results reflect only associations between the pandemic and adherence to diabetes treatment, and not causal relationships. Also, the SCI-R was applied only 1 month after the start of social distancing. Considering that the effects of social distancing on adherence can be time dependent, it is possible that the scores would worsen over time. The SCI-R was originally validated for self-administration; therefore, its administration by telephone could introduce a potential measurement bias. Finally, the difference in the time of questionnaire administration between the social distancing and control groups may also have interfered with the results. Nevertheless, to the best of our knowledge, this is the first study to assess the impact of social distancing on treatment-adherence parameters in diabetes.

Reduced availability of multidisciplinary teams and increased difficulty in obtaining medical care during the pandemic may directly interfere with treatment adherence in the future. In addition, studies have shown an increase in psychological and eating disorders during quarantine, which may have an even greater long-term impact in patients with diabetes, especially by negatively impacting adherence to recommended diabetes self-care behaviors (13-14). Further studies are warranted to better understand the impact of home confinement on adherence parameters in diabetes.

Author contributions: Debora Wilke Franco – conceptualization, methodology, writing-original draft preparation. Janine Alessi – conceptualization, methodology, software, data curation, writing-original draft preparation. Alice S. Becker – methodology, investigation. Bibiana Brino do Amaral – methodology, investigation. Giovana B. de Oliveira – methodology, investigation, writing-original draft preparation. Beatriz D. Schaan – conceptualization, validation, supervision, writing, reviewing and editing. Gabriela H. Telo – conceptualization, data curation, writing-original draft preparation, supervision. We declare that all authors are aware of the submission of this manuscript and have allowed to place their name as authors. Debora W. Franco is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Ethics: the project was approved by the institution's research ethics committee under number 4.029.368 and all authors signed the term of responsibility for data use.

Prior presentation: no prior presentation of the data presented has been made.

Data sharing: the data collected for the study will be available for 2 years after publication of the article.

Disclosure: no potential conflicts of interest relevant to this article were reported.

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Archives of Endocrinology and Metabolism
OFFICIAL JOURNAL OF THE BRAZILIAN SOCIETY OF ENDOCRINOLOGY AND METABOLISM

AE&M AWARDS 2020

Since 2001, the *Archives of Endocrinology and Metabolism* (AE&M) and the Brazilian Society of Endocrinology and Metabology award the "AE&M award Professor Thales Martins" and "AE&M award Professor Waldemar Berardinelli" to the young Brazilian researcher with the best original reports published in the AE&M in the areas of basic, translational science and clinical practice.

The commission was made up by members of the Editorial Board: Marcello D. Bronstein, Ana Luiza Maia, Beatriz D'Agord Schaan, Bruno Ferraz de Souza, Bruno Halpern, Francisco Bandeira, Fernanda Vaisman, Fernando M. A. Giuffrid, João Roberto Maciel Martins, Melanie Rodacki, Monica R. Gadelha, Nina Rosa C. Musolino, Poli Mara Spritzer, Ricardo Meirelles, Sandra Roberta Gouvea Ferreira Vivolo, Simone Van de Sande Lee, Tânia S. Bachega who analyzed the reports published in the AE&M, volume 64, 2020. The award ceremony is going to take place during the 35th Brazilian Congress of Endocrinology and Metabology (CBAEM) on September 10, 2021, completely virtual.

AE&M AWARD PROF. THALES MARTINS

"High-sugar diet leads to obesity and metabolic diseases in *ad libitum*-fed rats irrespective of caloric intake"

Authored by Daiane Teixeira de Oliveira, Isabela da Costa Fernandes, Graziele Galdino de Sousa, Talita Adriana Pereira dos Santos, Nívia Carolina Nogueira de Paiva, Cláudia Martins Carneiro, Elísio Alberto Evangelista, Natália Rocha Barboza, Renata Guerra-Sá.
Arch Endocrinol Metab. 2020;64(1):71-81

AE&M AWARD PROF. WALDEMAR BERARDINELLI

"The impact of minimal extrathyroidal extension in the recurrence of papillary thyroid cancer patients"

Authored by Maria Fernanda Ozorio de Almeida, Júlia Soares Couto, Ana Luiza Trevizani Ticly, Vivian Cenize Guardia, Marilia Martins Silveira Marone, Nilza Maria Scalissi, Adriano Namo Cury, Carolina Ferraz, Rosália do Prado Padovani. Arch Endocrinol Metab. 2020;64(3):251-6

Instructions for authors

1. EDITORIAL POLICIES

The Archives of Endocrinology and Metabolism - AE&M is a peer-reviewed and open access journal whose mission is publishing and disseminating original research in the fields of endocrinology, diabetes and metabolism. The journal publishes the following categories of articles: Original Article, Review Article, Brief Communications, Guidelines and Consensus, Case Report and Letter to the Editor.

The AE&M follows the recommendations of the International Committee of Medical Journal Editors (ICMJE – http://www.icmje.org/), the Committee on Publication Ethics (COPE – https://publicationethics.org/), of the Council of Science Editors (CSE – https://www.councilscienceeditors.org/), of the World Association of Medical Editors (WAME – http://www.wame.org/) and the best practices manual of the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (http://www.fapesp.br/boaspraticas/).

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AE&M endorses the concept of people-first language. The language, in all publications, should be placed first on the person and then the disease, as the following example: subject with obesity instead of obese subject, subject with diabetes instead of diabetic subject. The same should apply to other diseases, for example: not using acromegalic, osteoporotic, hypertensive, among others. Check your text before posting to avoid unnecessary resubmission delays. For more information, see: https://bit.ly/3z4YdPX.

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Category	Manuscripts (Words)	Tables/ Figures (Number)	References (Number)
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Brief Communication	1,500	2	30
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