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''Old in Sorrow''
Van Gogh
1890

"Nobody enters the same river a second time, because when that happens you are no longer the same, just like the water that will already be different"

"Nothing is permanent except change"

"Everything flows. Everything is in motion and nothing lasts forever"

HERACLITUS

EDITORIAL

AGING WITH QUALITY, DIGNITY AND HEALTH

Being old and passively accepting old aging as a deplorable and evil state has a lot to do with your mental state of mind and your way of facing this phase of life.

Some tips of how to live this phase of life not feeling old, but well and better. Also, taking care of your physical, psychological and spiritual well-being.

1 – Start practicing physical exercises in the best way you like. It could be a short walk, going to the gym, swimming and walks in the parks. Start slowly, but practice constantly. That way you will avoid muscle atrophy, which causes serious pain and locomotion problems. Always get out of your comfort zone.

2 – Avoid hunched postures and don´t keep your head down, looking at the floor and dragging your feet. That makes anyone feel or looks like an “old man”. Have positive attitudes and always look forward to the horizon.

3 – Maintain constant mental and intellectual activities. Develop new skills. Learn about new facts, learn a new language, make crafts, paintings, learn a new song and listen to music you like. Go out dancing. Seek to evolve in every way. Take courses of any nature and reinforce what you've learned throughout your life

4- Avoid isolation. Get out of the house, get out of the couch. Stay connected with groups and family. Maintain friendships and always try to stay in touch with friends. Have fun. Traveling is a great way to feel alive and happy. Travel as much as you can. Make yourself useful to people in general. Be altruistic and participate in volunteer work. "Who is supportive, is not lonely".

5 - Be tidy and well dressed as if you were about to leave the house. Only use comfortable pajamas and clothes for sleeping. When getting up, put on different clothes. A little advice for young people: Give your seniors running shoes and sports clothes. Avoid pajamas and slippers. This type of gifts are invitations not to leave the house.

6 – No one likes to hang out with whining people. So, don't complain too much, don't be “bitter” and don't complain about life. Playing the victim is never a good way to life. Stop living in the past. Always have optimistic attitudes related to the present and the future. Be kind to everyone. Look on the bright side of things and be grateful for the life you have.

7 – One of the main causes of youth is to always smile and keep a good mood.

8 – Get vaccinated with the necessary vaccines. Treat your illnesses correctly and visit your doctor regularly.

9 – Be organized, keeping objects in the usual places. Make them easy to find and be disciplined in all your daily activities.

10 – The main causes of premature aging and serious diseases are smoking and alcoholism, that should be avoided.

11 – Eat well and properly. Avoid reheating the same food for several days. Find some new recipes and make cooking a fun time.

12 – Have a goal of life. Think about new projects whether small or large, such as travelling, walking, keep studying. We always have something new to learn. think about getting a new house. Avoid the routine. Routine is the rust of the soul.

13 – Try to sleep well. Quality sleep rejuvenates and prevents several diseases. “Who does not sleep well, the next day is nobody”.

14- “Let go of the ties of the past, live intensely in the present and plan for the future”.

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... *"that's life, a second that disappears in time*
We all have our moment
And after it only oblivion"

"Caco velho" de Ary Barroso

LUIZ ANTONIO SÁ

GERIATRICIAN AND SPECIALIST IN MEMORY

FACULDADE EVANGÉLICA MACKENZIE DO PARANÁ - BRAZIL

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ORIGINAL ARTICLE

ANALYSIS OF BODY MASS INDEX (BMI) AT BREAST CANCER DIAGNOSIS AND OF REPORTED BMI FROM THE PAST DECADES OF LIFE

ANÁLISE DO ÍNDICE DE MASSA CORPORAL (IMC) AO DIAGNÓSTICO DO CÂNCER DE MAMA E DO IMC REPORTADO DE DÉCADAS ANTERIORES DE VIDA

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Key-words: Câncer da Mama, Índice de Massa Corporal, Diagnóstico
Descritores: Breast Neoplasms, Body Mass Index, Diagnosis

Abstract

Introduction: Breast cancer is the most common malignancy among women and the one with the highest mortality. Environmental, behavioral and biological factors have been implicated in the genesis of this neoplasm. **Objective:** To evaluate the relationship between body mass index (BMI) at diagnosis and the menstrual condition of patients and the molecular subtype of invasive ductal breast carcinoma, as well as to relate these same parameters to the variation in BMI over the decades of life. **Material and methods:** Cross-sectional study in patients undergoing systemic treatment for breast cancer between 2015 and 2020. Data were collected from medical records, as well as, prospectively, reported by patients, weight and height information of the past decades of life. **Results:** 56 patients were included, 71.4% premenopausal and 28.6% postmenopausal. There was no statistical difference in mean BMI at diagnosis according to menstrual condition, p=0.15. There was a statistical difference in median BMI at diagnosis between luminal B (25.3) compared to HER2+ (22.7), p=0.01. There was a higher mean BMI over the decades for pre- and postmenopausal women (p<0.001), as well as a greater increase in BMI, in the same period, for luminal women compared to HER2+ (p=0.003). Even so, BMI variation comparison among groups was similar when considering the menstrual condition (p=0.33) and when considering the molecular cancer subtype (p=0.41). **Conclusion:** The authors conclude that BMI at diagnosis was similar between pre- and postmenopausal patients, but higher for patients with luminal B compared to HER2+. Over the decades, there has been significant weight gain in patients regardless of menstrual condition at the time of breast cancer diagnosis, and in those with tumors of the luminal molecular subtype, this increase was greater when compared to HER2+. **Endocrinol diabetes clin exp 2022 / 2327-2334.**

Resumo

Introdução: O câncer de mama é a neoplasia maligna mais comum entre as mulheres e a de maior mortalidade. Condições e hábitos de vida têm sido implicados na gênese desta neoplasia. **Objetivo:** Avaliar a relação do índice de massa corporal (IMC) ao diagnóstico com a condição menstrual das pacientes e com o subtipo imunohistoquímico do carcinoma ductal invasor da mama, bem como, relacionar estes mesmos parâmetros com a variação do IMC ao longo das décadas de vida. **Material e métodos:** Estudo transversal, em pacientes submetidas a tratamento sistêmico para o câncer de mama entre 2015 e 2020. Foram coletados dados dos prontuários, bem como, de forma prospectiva, reportados pelas pacientes, informações de peso e altura ao longo das décadas de vida. **Resultados:** Foram incluídas 56 pacientes, sendo, 71,4% pré-menopausadas e 28,6% pós-menopausadas. Não houve diferença estatística da média de IMC ao diagnóstico de acordo com a condição menstrual, p=0,15. Houve diferença estatística da mediana de IMC ao diagnóstico entre as luminais B (25,3) na comparação com as HER2+ (22,7), p=0,01. Houve uma maior média de IMC ao longo das décadas para as pré e para as pós-menopausadas (p<0,001), assim como um maior incremento de IMC, neste mesmo período, para as luminais na comparação com as HER2+ (p=0,003). Mesmo assim, a variação de peso na comparação entre grupos foi igual tanto ao considerar a condição menstrual (p=0,33), como ao considerar o subtipo imunohistoquímico do tumor (p=0,41). **Conclusão:** Os autores concluem que o IMC ao diagnóstico foi semelhante entre pacientes na pré e pós-menopausa, mas superior para as pacientes com tumores luminais B na comparação com as HER2+. Ao longo das décadas, existe significante ganho de peso das pacientes independente da condição menstrual ao diagnóstico do câncer de mama, e naquelas com tumores do subtipo molecular luminal, este aumento foi maior quando comparado às HER2+. **Endocrinol diabetes clin exp 2022 / 2327-2334.**

INTRODUCTION

Breast cancer is the most common cancer in women worldwide, excluding non-melanoma skin cancer, with roughly 2.3

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*The authors have contributed equally in the study conduction, in performing statistical analysis and in results compilation.
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million new cases in the world estimated in 2020, representing a quarter of the malignant neoplasms in women (1). The National Cancer Institute (INCA) estimated 66,280 new cases in Brazil in 2021, corresponding to an incidence of 61.61 cases per 100,000 women, approximately (1). The risk of developing breast cancer increases with the age of the patient, with the period between 50-69 years old (postmenopausal) being the most frequently affected by the disease (2).

Ductal carcinoma is the most common malignant tumor of the breast. There are several immunohistochemical or molecular subtypes of breast cancer, which are categorized according to the expression or not of hormone receptors (estrogen and progesterone) or the human epidermal growth factor receptor 2 (HER2). Environmental, behavioral and biological factors are cited as risk factors for this disease; among them, diet, physical activity and weight, often represented by body mass index (BMI), are usually reported (3). BMI usually represents a way of measuring obesity, even though in a flaw manner, and is calculated as the ratio of body weight in relation to height squared, with the normal range between 18.5 and 24.9 for adult population (4).

Recent studies have shown that overweight and obesity are associated with a higher risk of developing several malignant tumors, such as in the cardia, distal esophagus, colon and rectum, endometrium, ovary and breast (5). Obesity is consistently associated with an increased risk of postmenopausal breast cancer in several studies (6,7,8). Adipose tissue produces inflammatory cytokines and mediators that create a suitable biological condition for malignant tumor development and progression (9).

The relationship between overweight and obesity and breast cancer risk is paradoxical. The literature has shown an inverse relationship between BMI and premenopausal breast cancer risk, but a direct relationship between BMI and postmenopausal breast cancer risk (10). A robust meta-analysis, which included more than 2.5 million women, with 7,930 women with premenopausal breast cancer and 23,909 women with postmenopausal breast cancer, demonstrated that the risk of breast cancer in premenopause period is reduced by 8% for each 5 kg/m2 increase in BMI, but that there is a relative risk of 1.12 (95% CI, 1.08-1.16) of postmenopausal breast cancer, for the same BMI increment (11). Another study showed that obesity is responsible for a 30-50% raise in breast cancer cases in postmenopausal women, while an increase of 5 kg/m2 in BMI can increment the risk of developing breast cancer by 9- 31% (12).

The association of weight in childhood, adolescence and early adulthood with breast cancer has been studied. One study, evaluated in England, prospectively, the relationship between adiposity throughout life and the risk of postmenopausal breast cancer (13). This study of 342,079 postmenopausal women included 15,506 cases of breast cancer and reported that although the increased risk of postmenopausal breast cancer is related to BMI gain at age 60, women with higher BMI at childhood and at early adulthood, showed a reduced risk of postmenopausal breast cancer (13). This finding is in agreement with some authors (14,15), but in disagreement with others (16,17). In the literature, the evaluation of weight variation throughout life and its association with breast cancer is scarce, as it would require a large, prospective study, evaluating in a longitudinal manner, for decades, the variation in body composition, since birth, of lifestyle habits, diet, practice of physical activity and comorbidities.

Having said that, this study aim to explore, in a sample of patients with breast cancer, the relationship between BMI at diagnosis and BMI variation over decades of life, and the menstrual condition of the patients and the molecular subtype of invasive ductal breast carcinoma.

MATERIAL AND METHODS

Cross-sectional study, with ambispective data collection, which was approved by the Human Research Ethics Committee of Faculdade Evangélica Mackenzie do Paraná, Brazil (FEMPAR). Patients were selected if they were aged more than 18 years, treated for breast cancer between 2015-2020 at the Clinical Oncology Center (CEON) of Hospital Universitário Evangélico Mackenzie (HUEM), and they should had phone contacts available in clinical records.

Among patients in whom telephone contact was possible for prospective data collection, the medical records were used to provide retrospective clinical information. Therefore, a questionnaire was applied that included gynecological and obstetrical history, as well as the reported weight and height for each decade of life from the age of 20, in order to calculate BMI variation.

Clinical data such as pathology, tumor stage and treatment were obtained by consulting clinical charts of patients who answered the questionnaire. Tumor stage was classified using the guideline of the *TNM (Tumor – Node – Metastasis) 8th Edition of the American Joint Committee on Cancer (AJCC)* (18) and the Guideline 5.2021 of the *National Comprehensive Cancer Network* (NCCN) (19). The cancer immunohistochemistry was classified according to the Clinical Practice Guidelines of the European Society for Medical Oncology (ESMO), 2019 (20).

The data obtained were stored at REDCap® Platform, version 11.1.15, and the statistical analysis were done using SPSS® Software, version 21. The Kolmogorov- Smirnov and the Shapiro-Wilk tests were done to determine whether the data was normally distributed; if so, mean BMI was used for comparison, conversely, if not, than the median BMI was the reference. The comparative analysis of categorical data was performed using Fisher and Chi-square tests. Comparison of numerical data was performed using Mann-Whitney and unpaired Student's t-tests. The association analysis of BMI and menstrual condition was performed using the Student's t-test and with different molecular cancer subtype was performed using Kruskal-Wallis and Mann-Whitney tests. Comparisons of BMI variation over decades stratified by menstrual condition and by molecular cancer subtype were done using mixed model analysis, fixed effects. The significance adopted was 5%.

RESULTS

A total of 216 patients met the inclusion criteria, and telephone contact was possible in 56 (25.9%). Among these, 40 (71.4%) patients were premenopausal at the time of breast cancer diagnosis, while 16 (28.6%) were postmenopausal - **Table 1**. For patients who had the diagnosis in the postmenopause period, the mean age at menopause was 46.8 years, with a standard deviation of 4.6.

The mean age at menarche of the patients in the study was 12.7 years, with a standard deviation of 1.8. **Table 1** shows that 52 (92.9%) patients had children before the diagnosis of breast cancer, with a uniform distribution of their number. The age at first pregnancy was, on average, 22.3 years old, with a standard deviation of 5.9 years. Of those who were pregnant, 86.5% had history of breastfeeding, and of these, more than 50% did so for more than 12 months.

The mean age at breast cancer diagnosis was 46.4 years, with a standard deviation of 6.4 years, with a minimum age of 28 years and a maximum of 59 years. The average age of premenopausal and postmenopausal women was 43 years and 52.7 years, respectively. In the analysis of immunohistochemistry, staging and treatment, a lesser number of patients had valid data in their medical records, and according to **Table 1**, there was a predominance of luminal B (36.4%) and luminal A (27.3%) breast cancer molecular subtypes. The cancer staging at diagnosis was initial in the majority of patients (69.9%). In terms of treatment, more than 90% of the sample underwent at least surgery and/or chemotherapy and/or hormone therapy.

Table 1. Descriptive analysis of the sample

Variables	n (%)
Menstrual condition at breast cancer diagnosis	
Premenopausal	40 (71.4)
Postmenopausal	16 (28.6)
History of gestation	
Yes	52 (92.9)
No	4 (7.1)
Number of children if history of gestation	
1	16 (30.8)
2	19 (36.5)
≥ 3	17 (32.7)
Breastfeeding if history of children	
Yes	45 (86.5)
No	7 (13.5)
Breastfeeding time for who had breastfed	
1 to 6 months	8 (17.8)
6 to 12 months	13 (28.9)
> 12 months	24 (53.3)
Immunohistochemistry*	
Luminal B	20 (36.4)
Luminal A	15 (27.3)
Her2+	12 (21.9)
Luminal-like	5 (9)
Triple negative	3 (5.4)
Cancer staging (NCCN)#	
Initial	37 (69.9)
Locally Advanced	11 (20.7)
Metastatic	5 (9.4)
Treatment performed*$	
Surgery	51 (94.4)
Chemotherapy	51 (94.4)
Hormone therapy	50 (92.6)
Radiotherapy	45 (83.3)
Monoclonal antibody	12 (22.2)

* n=55, due to missing data for 1 patient; # n=53, due to missing data for 3 patients; $ more than 1 treatment was done

Table 2 shows the categorical division of body mass index (BMI) of the patients according to their menstrual condition. It depicts that 7.1% of the sample were underweight at diagnosis, while most of the patients (53.5%) were in the normal weight range. Of the total, 28.5% of the patients were overweight and 10.7% were obese. Among the premenopausal women, the majority (57.5%) were normal weight, as well as the postmenopausal women (43.7%).

Table 2. Correlation of body mass index (BMI) at diagnosis whit menstrual condition of the patients

BMI at diagnosis	Menstrual condition - n (%)	
	Premenopausal	*Postmenopausal*
<18.5 (underweight)	3 (7.5)	1 (6.2)
from 18.5 to < 25 (normal weight)	23 (57.5)	7 (43.7)
from 25 to < 30 (overweight)	11 (27.5)	5 (31.2)
≥30 (obese)	3 (7.5)	3 (18.7)

The mean BMI at breast cancer diagnosis for patients diagnosed in the premenopause period was 23.5, with a standard deviation of 3.4. In comparison, patients who were diagnosed in the postmenopause period had a mean BMI of 25.1, with a standard deviation of 5.1. These results can be seen at **Figure 1**. When comparing mean BMI at breast cancer diagnosis between the two menstrual condition groups, there was not a statistical difference (p=0.15).

Figure 1. Distribution of mean body mass index (BMI) at breast cancer diagnosis according to menstrual condition

When comparing BMI variation throughout decades of life according to menstrual condition, it can be seen that there is a tendency towards weight gain, both among pre- and postmenopausal women at the time of cancer diagnosis. This difference can be seen at twenty years of age, when compared to the decades later (p<0.001). Despite this, there was not a difference in weight variation along decades of life between pre- and postmenopausal women (p=0.33) – **Table 3.**

Table 3. Mean BMI variation throughout decades of life according to menstrual condition at breast cancer diagnosis

Decade of life	Menstrual condition	n	*Mean (SD)
20 years	Premenopausal	40	**20.1 (2.4)**
	Postmenopausal	16	**21.5 (4.4)**
30 years	Premenopausal	40	21.8 (2.4)
	Postmenopausal	16	24 (5.13)
40 years	Premenopausal	32	23 (2.8)
	Postmenopausal	16	24.9 (5)
50 years	Premenopausal	9	24.7 (5.1)
	Postmenopausal	12	26.5 (5.8)
60 years	Premenopausal	1	24 (-)**
	Postmenopausal	1	25 (-)**

*Mean and standard deviation (SD) of BMI.
**There was not a SD.

When analyzing the immunohistochemistry subtype, the mean age of patients with breast cancer luminal B subtype or luminal B + luminal-like was 45.4 years, while of luminal A was 48.6 years, of HER2+ was 45.4 years and of triple negative was 48.6 years. The mean age of the grouped luminals (A+B+like) was 46.5 years. Among premenopausal women, the majority, 32 patients (80%) were luminal, 7 (17.5%) were HER2+ and 1 (2.5%) was triple-negative, while among the postmenopausal women, 8 patients (53.4%) were luminal, 5 (33.3%) were HER2+ and 2 (13.3%) were triple negative.

Analyzing the BMI at diagnosis of patients according to immunohistochemistry subtype, higher median averages can be seen for those patients with hormone receptor positive tumors – luminal B, when compared to HER2+ (p=0.01). The median BMI for luminal A patients was 22; the lowest value was 19, and the highest BMI value was 34. For luminal B group the median was 25; the lowest value was 21, while the highest value was 31. The HER2+ group had a median BMI of 23; the minimum BMI value was 18, while the maximum was 27. This distribution is illustrated in **Figure 2**.

Figure 2. Distribution of median body mass index (BMI) at breast cancer diagnosis according to breast cancer immunohistochemistry subtype

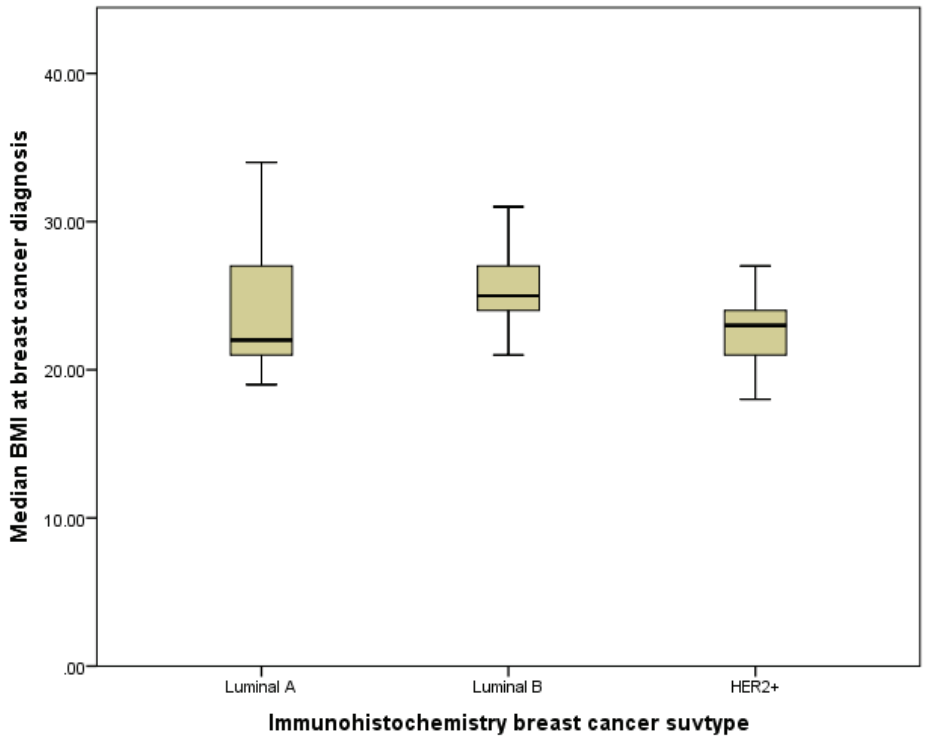


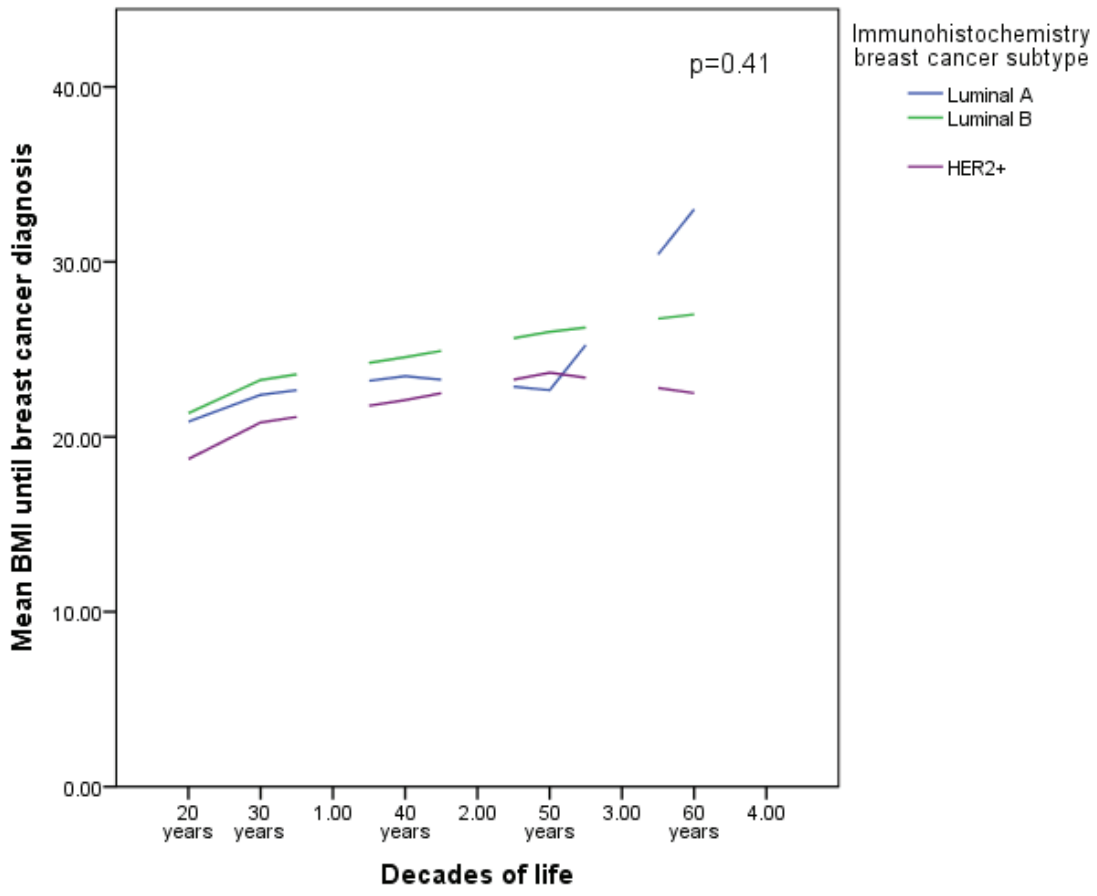
Figure 3 and **table 4** illustrate the mean BMI variation over the lifetime of patients who had luminal A, luminal B and HER2+ tumors. It can be seen that in addition to an increase in weight over the decades of life in the 3 subtypes (p<0.001), there was also a clear difference in the average weight of the luminal A and B, when compared to HER2+ subtype (p=0.003) – **Table 4**. On the other hand, weight variation over the decades was similar when comparing the three groups (p=0.41) – **Figure 3.**

Table 4. Mean BMI variation throughout decades of life according to breast cancer immunohistochemistry (IHC)

Decade of life	IHC	n	*Mean (SD)
20 years	Luminal A	15	20.8 (3.6)
	Luminal B	20	21.3 (3)
	HER2+	11	**18.7 (2.3)**
30 years	Luminal A	15	22.4 (3.4)
	Luminal B	20	23.2 (3.5)
	HER2+	11	**20.8 (1.9)**
40 years	Luminal A	15	23.4 (4.3)
	Luminal B	20	24.5 (3.7)
	HER2+	10	**22.1 (2.2)**
50 years	Luminal A	9	22.6 (4)
	Luminal B	13	26 (3)
	HER2+	6	**23.6 (2)**
60 years	Luminal A	1	33 (-)**
	Luminal B	1	27 (-)**
	HER2+	2	**22.5 (3.5)**

*Mean and standard deviation (SD) of BMI.
**There was not a SD.

Figure 3. Graphic representation of mean body mass index (BMI) over lifetime according to breast cancer immunohistochemistry subtype



DISCUSSION

The results of the present study show an increase in mean BMI over the decades, regardless of the menstrual condition at the time of breast cancer diagnosis. In addition, patients with hormone receptor positive tend to have a median BMI at diagnosis of breast cancer higher than HER2+, mainly luminal B. There was an increase in mean BMI over the decades for all subtypes, with a greater increment of the mean BMI of the luminal A and B in comparison with the HER2+. Even so, mean BMI variation over the decades was similar both in the comparison between pre- and postmenopausal women and among the cancer immunohistochemistry subtypes.

The present study had most of the sample (71.4%) of premenopausal patients at breast cancer diagnosis. Gaudet et al. similarly showed that most of the patients in their study were premenopausal at breast cancer diagnosis (21). Conversely, Felden and Figueiredo, showed the majority of their study sample in the postmenopause period (22). Regarding the number of children and duration of breastfeeding, this study found similar results to those found by Gaudet et al. (21) and Felden and Figueiredo (22).

In terms of BMI at breast cancer diagnosis, the present study found a greater number of patients (53.5%) in the normal weight range. This result differs from that found by Silva and Figueiredo (23), who showed a mean BMI for patients with breast cancer of 29.2, which is in the overweight range, a similar finding described by Turkoz et al. (24), that verified a great number of their sample in the overweight range. Despite this, the present result converges to that found by Gaudet et al. (21), who showed that 69.4% of the patients was in the normal weight range. The present study may have presented the majority of patients in the normal weight range, possibly due to the lower mean age of postmenopausal women. Although it is possible to see that, numerically, BMI at diagnosis of postmenopausal women is greater than that of premenopausal women, there is no statistical significance, possibly, in part, due to the small sample size and also, as previously mentioned, to the low mean age of postmenopausal women in this study.

When analyzing BMI variation of the patients throughout their decades of life, it is possible to notice that patients diagnosed in the postmenopause period had consistently higher BMI means throughout the decades, although there was no significant difference when compared to premenopausal women. In addition, we can evidence a general weight gain for all patients. This finding complements what was demonstrated by Ellingjord-Dale et al. (25), that patients diagnosed with breast cancer in the pre- or in the postmenopause period had an increased risk of developing the disease when they had gained more than 10 kg throughout their lives. The same author concluded that for postmenopausal women, weight gain greater than 10 kg was associated with a 42% increase in breast cancer risk.

The results of the present study also showed that patients with hormone receptor positive tumors, mainly luminal B, had higher median BMI at diagnosis. It was also possible to show that the mean BMI of the luminal subtype was higher when compared to the mean BMI of the HER2+ throughout decades of life, with a statistically significant difference. Despite this, BMI variation throughout life was similar among the groups, regardless of the immunohistochemical cancer subtype. The study by Kawai et al. (26) showed that in the group of HER2+ patients, 62% had a BMI lower than 25, that is, in the normal weight range. Regarding the analysis of BMI throughout life, Ellingjord-Dale et al. (25) demonstrated that a weight gain greater than 10 kg carried a 46% increased risk for patients to develop hormone receptor positive tumors (luminal). As reviewed by Neuhouser et al. (27), this finding can be understood as the peripheral aromatization of steroids into estrogens, which leads to the development of hormone receptor positive tumors. The present study shows that overweight in luminal tumors is not related to older age, since most luminal patients were premenopausal and the average age at diagnosis of patients with breast cancer luminal subtype was 46.5 years, almost similar to the age of patients with breast cancer subtype HER2+, which was 45.4 years. Although it was not the objective of this study, there seems to be a relationship between weight gain over the decades of life, increased BMI at breast cancer diagnosis and hormone receptor positive tumors (so-called luminal), and that this relationship does not seem to be strictly related to the weight gain inherent to aging.

The main weakness of the present study is the small sample size of patients with whom telephone contact was achieved. In addition, the numerical disproportion between pre- and postmenopausal women and the low average age (52.7 years) of postmenopausal women, since the oldest patient in the study was 59 years old at breast cancer diagnosis, can also be all cited as potential flaws of the present study. This may be due to the fact that the initial selection of patients was based on the entry registration in the chemotherapy sector of the institution, therefore, those who underwent chemotherapy or hormone therapy were included. This ended up not allowing the inclusion of patients with no indication for hormone therapy or chemotherapy and the very elderly who had some contraindication for systemic treatment, which may have reduced the average age of postmenopausal women of the study. The limitations inherent to a retrospective study, such as the lack of complete clinical information in medical records, including compilation of comorbidities and lifestyle habits, has also to be considered. In addition, the limitations of BMI, which does not faithfully represent the body composition and distribution of lean and fat mass, and a potential flaw related to BMI from previous decades of life reported by patients which depends on a good and trustful memory have also to be considered as study weaknesses.

Even so, the present study brought important information, by obtaining mean BMI variation over decades of life reported in a prospective manner, in about a quarter of the study population, counting from the second decade of life onwards.

In conclusion, by demonstrating that there is a difference in mean BMI throughout decades of life in patients with hormone receptor positive breast cancer, who tend to be more overweight, this study leverages the possibility to validate, through prospective and larger studies, if this variation in weight over the decades really differs between luminal and non-luminal tumors. Having said that, the authors raise the possibility whether epidemiological measures of weight control throughout life could minimize years later, the impact on development of luminal subtype breast neoplasms. This cause-effect relationship lacks evidence and could possibly be verified through multicenter, longitudinal, long-term studies in the general female population.

CONCLUSION

The authors conclude that BMI at breast cancer diagnosis was similar between pre and postmenopausal patients, however it was higher for patients with ductal breast carcinoma of the luminal B subtype in comparison with HER2+. When analyzing mean BMI over the decades of life, there is a trend towards greater weight gain among postmenopausal women at the time of breast cancer diagnosis, despite the lack of statistical significance. Finally, BMI reported in previous decades of life was statistically higher for luminal subtype compared to the HER2+. Even so, mean BMI variation over the decades based on menstrual condition or on cancer molecular subtype was similar among groups.

References

1- Instituto Nacional de Câncer (INCA); 2023 Jan 10. Outubro Rosa - 2022: *Eu cuido da minha saúde todos os dias. E você?* Available from: **inca.gov.br**

2- Dunneram Y, Greenwood DC, Cade JE. Diet, menopause and the risk of ovarian, endometrial and breast cancer. **Proc Nutr Soc**. 2019; 78(3): 438-448.

3- Rojas K, Stuckey A. Breast cancer epidemiology and risk factors. **Clin Obstet Gynecol**. 2016; 59: 651-672.
4- Silva TM, Vaz JS, Mola CL, Assunção MCF, Tovo-Rodrigues L. Prevalências de obesidade em zonas rurais e urbanas no Brasil: Pesquisa Nacional de Saúde, 2013. **Rev Bras Epidemiol**. 2019; 22(1).
5- World Cancer Research Fund (WCRF). Continuous Update Project: 2016. London, UK: WCRF International; 2016.
6- White AJ, Nichols HB, Bradshaw PT, Sandler DP. Overall and central adiposity and breast cancer risk in the sister study. **Cancer**. 2015; 121:3700-3708.
7- van den Brandt PA, Spiegelman D, Yaun SS, Adami HO, Beeson L, Folsom AR, Fraser G, Goldbohm RA, Graham S, Kushi L, Marshall JR, Miller AB, Rohan T, Smith-Warner SA, Speizer FE, Willett WC, Wolk A, Hunter DJ. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. **Am J Epidemiol**. 2000; 152:514-527.
8- Li CI, Malone KE, Daling JR. Interactions between body mass index and hormone therapy and postmenopausal breast cancer risk (United States). **Cancer Causes Control**. 2006; 17:695-703.
9- Gilbert CA, Slingerland JM. Cytokines, obesity, and cancer: new insights on mechanisms linking obesity to cancer risk and progression. **Annu Rev Med**. 2013; 64: 45-57.
10- Munsell MF, Sprague BL, Berry DA, Chisholm G, Trentham-Dietz A. Body mass index and breast cancer risk according to postmenopausal estrogen-progestin use and hormone receptor status. **Epidemiol Rev**. 2014; 36:114–36.
11- Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. **Lancet**. 2008;371:569-578.
12- Waks A.G., Winer E.P. Breast cancer treatment: a review. **J Am Med Assoc**. 2019; 321:288-300.
13- Yang TO, Cairns BJ, Pirie K, Green J, Beral V, Floud S, Reeves GK. Body size in early life and the risk of postmenopausal breast cancer. **BMC Cancer**. 2022; 22:232.
14- Baer HJ, Tworoger SS, Hankinson SE, Willett WC. Body fatness at young ages and risk of breast cancer throughout life. **Am J Epidemiol**. 2010; 171:1183–94.
15- Fagherazzi G, Guillas G, Boutron-Ruault MC, Clavel-Chapelon F, Mesrine S. Body shape throughout life and the risk for breast cancer at adulthood in the French E3N cohort. **Eur J Cancer Prev**. 2013; 22:29–37.
16- Canchola AJ, Anton-Culver H, Bernstein L, Clarke CA, Henderson K, Ma H, Ursin G, Horn-Ross PL. Lifetime body size and the risk of postmenopausal breast cancer subtypes in the California Teachers Study cohort. **Cancer Causes Control**. 2012; 10.1007/s10552-012-9897-x.
17- Horn-Ross PL, Canchola AJ, Bernstein L, Neuhausen SL, Nelson DO, Reynolds P. Lifetime body size and estrogen-receptor-positive breast cancer risk in the California Teachers Study cohort. **Breast Cancer Res.** 2016;18:132.
18- Kim J-Y, Lim JE, Jung HH,Cho SY,Cho EY, Lee SK, Yu JH, Lee JE, Kim SW, Nam SJ, Park YH, Ahn JS, Im Y-H. Validation of the new AJCC eighth edition of the TNM classification for breast cancer with a single-center breast cancer cohort. **Breast Cancer Res Treat**. 2018; 171(3):737-745.
19- National Comprehensive Cancer Network (NCCN), 2021. Available **from: nccn.org/guidelines**
20- Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, Zackrisson S, Senkus E. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. **Ann Oncol**. 2019; 30(8):1194-1220.
21- Gaudet MM, Press MF, Haile RW, Lynch CF, Glaser SL, Schildkraut J, Gammon MD, Thompson WD, Bernstein JL. Risk factors by molecular subtypes of breast cancer across a population-based study of women 56 years or younger. **Breast Cancer Res Treat.** 2011; 130(2):587–97.
22- Felden JBB, Figueiredo ACL. Distribuição da Gordura corporal e câncer de mama: um estudo de caso-controle no Sul do Brasil. **Cien Saude Colet**. 2011;16(5):2425–33.
23- Silva LM, Figueiredo JA. Metabolic syndrome and risk of cardiovascular diseases in female breast cancer survivors. **Int J Cardiovasc Sci**. 2021; 34(4).
24- Turkoz FP, Solak M, Petekkaya I, Keskin O, Kertmen N, Sarici F, Arik Z, Babacan T, Ozisik Y, Altundag K. Association between common risk factors and molecular subtypes in breast cancer patients. **Breast**. 2013; 22(3):344–50.
25- Ellingjord-Dale M, Christakoudi S, Weiderpass E, Panico S, Dossus L, Olsen A, Tjønneland A, Kaaks R, Schulze MB, Masala G, Gram IT, Skeie G, Rosendahl AH, Sund M, Key T, Ferrari P, Gunter M, Heath AK, Tsilidis KK, Riboli E, et al. Long-term weight change and risk of breast cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. **Int J Epidemiol**. 2021; 50(6):1914–26.
26- Kawai M, Malone KE, Tang M-TC, Li CI. Height, body mass index (BMI), BMI Change, and the risk of estrogen receptor-positive, HER2-positive, and triple-negative breast cancer among women ages 20 to 44 years. **Cancer**. 2014; 120(10):1548–56.
27- Neuhouser ML, Aragaki AK, Prentice RL, Manson JE, Chlebowski R, Carty CL, Ochs-Balcom HM, Thomson CA, Caan BJ, Tinker LF, Urrutia RP, Knudtson J, Anderson GL. Overweight, obesity, and postmenopausal invasive breast cancer risk: a secondary analysis of the women´s health initiative randomized clinical trials. **JAMA Oncol**. 2015; 1(5):611-21.

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ORIGINAL ARTICLE

RESEARCH PAPER ON AUTOIMMUNITY AND EPIGENETIC

IN SILICO STUDY OF METHYLATION IN CTLA-4 GENE PROMOTER REGION AS AN EPIGENETIC MECHANISM IN AUTOIMMUNE THYROIDITIS TRIGGERING SECONDARY TO HEPATITIS C

ESTUDO IN SILICO DA METILAÇÃO NA REGIÃO PROMOTORA DO GENE CTLA-4 COMO UM MECANISMO EPIGENÉTICO NO DESENCADEAMENTO DA TIREOIDITE AUTOIMUNE SECUNDÁRIA A HEPATITE C

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Key words: Epigenomics; Thyroiditis, Autoimmune; Hepatitis C; CTLA-4 Antigen.
Descritores: Epigenômica. Tireoidite autoimune; Hepatite C; Antígeno CTLA-4;

Abstract

Introduction: Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) is a crucial immune control point receptor that regulates T cell activation. Epigenetic mechanisms, such as DNA methylation and histone modifications, modulate DNA packaging in the nucleus and influence Gene expression. Autoimmune thyroiditis may be associated with hepatitis C virus (HCV) infection as well as the CTLA-4 Gene. **Objective:** To simulate in silico of the methylation in CTLA-4 gene promoter region as an epigenetic factor in triggering autoimmune thyroiditis by HCV. **Methods:** We analyzed by *in sílico* simulation the hypermethylation scenarios of the CTLA-4 Gene promoter region, aligning CTLA-4 and HCV sequences (genotypes 1, 2 and 3) through BLAST software - http://blast.ncbi.nlm.nih.gov/Blast.cgi, and identifying their methylated and unmethylated CpG sites. After the sequences obtained with the alignment of the methylation points by MultAlin program, the consensus sequences obtained were submitted to the BLAST similarity search. The GC content calculation and HCV annotation were performed using ENDMEMO (http://www.endmemo.com/bio/gc.php). The MethPrimer was used to identify and locate the methylation CpGi within the HCV genome. Results: The location of CTLA-4 on chromosome 2 and the alignment of the amino acid sequences are presented: CTLA-4 and HCV genotype 1, CTLA-4 and HCV genotype 2 and CTLA-4 and HCV genotype 3 are presented, as well as the methylation sites. **Conclusion:** In susceptible individuals, hypermethylation can promote reduced CTLA-4 expression and increases the risk of autoimmune thyroiditis in HCV-infected individuals. **Endocrinol diabetes clin exp 2022 / 2335-2340.**

Resumo

Introdução: O antígeno 4 citotóxico associado aos linfócitos T (CTLA-4) é um receptor crucial no controle imunológico que regula a ativação das células T. Mecanismos epigenéticos, como a metilação do DNA e modificações de histonas, modulam o empacotamento do DNA no núcleo e influenciam a expressão do gene. A tireoidite autoimune pode estar associada à infecção pelo vírus da hepatite C (HCV), bem como ao gene CTLA-4. **Objetivo:** Simular computacionalmente a metilação na região promotora do gene CTLA-4 como fator epigenético, desencadeando tireoidite autoimune pelo HCV. **Métodos:** Analisamos por simulação computacional os cenários de hipermetilação da região promotora do gene CTLA-4, alinhando as sequências do CTLA-4 e do HCV (genótipos 1, 2 e 3) através do programa BLAST - http://blast.ncbi.nlm.nih.gov/Blast.cgi e identificando seus locais CpG metilados e não metilados. Após as sequências obtidas com o alinhamento dos pontos de metilação pelo programa MultAlin, as sequências de consenso obtidas foram submetidas à busca de similaridade através do BLAST. O cálculo do conteúdo do GC e a anotação do vírus da hepatite C foram realizados usando o ENDMEMO (http://www.endmemo.com/bio/gc.php). O MethPrimer foi utilizado para identificar e localizar a metilação CpGi no genoma do HCV. Resultados: São apresentadas a localização do CTLA-4 no cromossomo 2 e o alinhamento das sequências de aminoácidos: CTLA-4 e HCV genótipo 1, CTLA-4 e HCV genótipo 2 e CTLA-4 e HCV genótipo 3 são apresentados, bem como os locais de metilação. **Conclusão:** Em indivíduos suscetíveis, a hipermetilação pode promover redução na expressão do CTLA-4 e aumenta o risco de tireoidite autoimune em indivíduos infectados pelo HCV.. **Endocrinol diabetes clin exp 2022 / 2335-2340.**

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INTRODUCTION

Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) a member of the immunoglobulin superfamily, is a crucial immune control point receptor that regulates T cell activation, has been identified as a risk factor for certain autoimmune diseases, becoming recognized as a risk agent for various T-cell-mediated autoimmune diseases (1,2).

The conception of epigenetics was first time suggested by Conrad Waddington in 1942 (3). Epigenetic occurrences lead to heritable alterations in gene manifestation another than the changes in DNA nucleotide sequences (4). The DNA-methylation is the most easily evaluated, and likely the most stead epigenetic characteristic, with important regulatory functions in animals. Epigenetic mechanisms, such as histone modifications or incorporation of variant histones, and DNA--methylation regulate DNA packaging in nucleus and act in gene expression, being capable design your own heredity via methylation of hemimethylated sites after DNA replication by preservation DNA-methylases (5,6). Histone transformations could be associated to reader-protein connection complexes to regulate gene expression, and favor to epigenetic heritance or to revert epigenetic marks in the chromatin (7). The histone deacetylation, DNA-hypermethylation or DNA-hypomethylation, and microRNAs in control of gene expression are epigenetic changes that can reciprocal action (8).

The interactions of complex virus-host-environment demonstrate that epigenetic mechanisms occur soon after hepatitis C virus (HCV) infection, once a new epigenetic condition recorded and eternalized in later mitosis, originating in the reprograming of the cell transcription (9).

A T-cell response is essential for response of HCV infection, and co-stimulatory molecules decrease T-lymphocyte responses by connection with CTLA-4 (10). The epigenetic mechanism of DNA-methylation in CTLA-4 Gene in autoimmune thyroiditis happens in region of 5'promoter regions with high density and principally results in transcriptional suppression, and this DNA--methylation has results a fault to establishment and maintenance immunologic non-responsiveness or tolerance to self-antigens (11). Thus, autoimmune thyroiditis may be associated with HCV infection as well as the CTLA-4 Gene.

The ease of the availability of vast amounts of sequence data, associate to advances in computational biology facilitates *in sílico* analysis of several molecular framework. The aim of our study was to *in sílico* simulate the methylation of the promoter region of CTLA-4 Gene as an epigenetic factor triggering autoimmune thyroiditis by HCV infection.

MATERIAL AND METHODS

We analyzed by *in sílico* simulation the methylation scenarios of the CTLA-4 Gene promoter region, aligning the CTLA-4 and HCV sequences (genotypes 1, 2 and 3), and identifying the methylation of genomic regions known as CpG islands (CpGi) sites.

Sequences analysis

We got the CTLA-4 and HCV sequences (genotypes 1, 2 and 3) through the FASTA format in the NCBI database (http://www.ncbi.nlm.nih.gov/), with the aim of identifying possible regions which methylations.

A sample of thyroid peroxidase [Homo sapiens] was acquired at NCBI database Accession: AAA61217.2 GI: 4680721.

The following HCV sequences were obtained: polyprotein [Hepatitis C virus genotype 1], Accession: NP_671491.1 GI: 22129793; polyprotein [Hepatitis C virus genotype 2], Accession: YP_001469630.1 GI: 157781213; and polyprotein [Hepatitis C virus genotype 3], Accession: YP_001469631.1 GI: 157781217.

A sample of CTLA-4 [Homo sapiens] was acquired at GenBank Accession: AAL07473.1 GI: 15778586.

After the sequences acquired with the alignment of the methylation points by MultAlin program, the consensus sequences acquired were submitted to the BLAST software similarity evaluation. MultAlin is a digital platform supported on an algorithm exploiting gradual paired alignment considering the relationships that may exist between some subsets of the sequels. The application add choices to group the intended output model and its dimension and colors besides configure individualized alignment specifications like the quantity of repetitions and gap punishment obligatory.

The guanine- cytosine (GC) content calculation and HCV annotation were performed using ENDMEMO (http://www.endmemo.com/bio/gc.php).

Primer nucleotide sequence of CTLA-4 was verified using Primer-BLAST in NCBI web site.

Analysis of methylation

The MethPrimer Express® Software v1.0 (Applied Biosystems) a web-based program which has been developed in 2002 by Li & Dahiya (12) was used to identify and locate the methylation CpGi within the HCV genome. MethPrimer is an online platform which provides a number of tools and databases to become possible the investigation of epigenetics and of DNA methylation, incorporating tools to project probes and primers for several bisulfite conversion based PCRs, predicting CpGi, and manipulating sequences.

The research involved data from the public domain, with no involvement of human beings, thus not needing to be submitted to the ethics committee.

RESULTS

The NCBI show that CTLA-4 has cytogenetic location in the 2q33.2, where is the long (q) arm of chromosome 2 at situation 33.2, and molecular localization in the base pairs 203,867,771 to 203,873,965 on chromosome 2 (**Figure 1**).

Figure 1. Location of CTLA-4 on chromosome 2
Source: NCBI.

Identification of CpGi

The default parameters as proposed by Deaton and Bird (13) were used to identify the potential CpGi. The parameters were as follows: length of DNA sequence > 300bp; CpG checked/CpG expected proportion > 0.6 and C+G% > 50%.

Thyroid peroxidase [Homo sapiens] - CpGi Prediction

In *silico* analysis and the primers for quantitative DNA methylation analysis of thryroid peroxidase, and the sequence alignment of CTLA-4 and Thyroid peroxidase [Homo sapiens] not revealed the presence of putative promoters and none CpGi (**Figure 2)**.

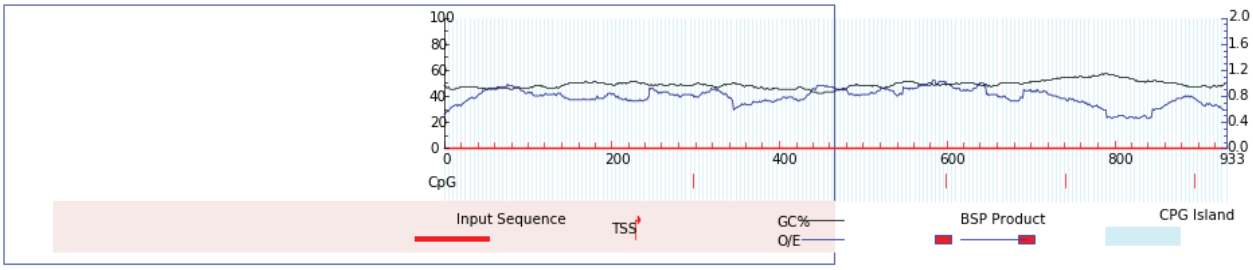

Figure 2. Prediction of methylation sites in the CpGi from Thyroid peroxidase [Homo sapiens]
Source: study result.

Hepatitis C virus genotype 1, complete genome - CpGi Prediction

The *in silico* analysis and the primers for quantitative DNA methylation analysis of HCV genotype 1 Gene, and the sequence alignment of the HCV genome and CTLA-4 demonstrated the existence of two putative promoters and two CpGi in the 5′ genomic region (**Figure 3 & 4**).

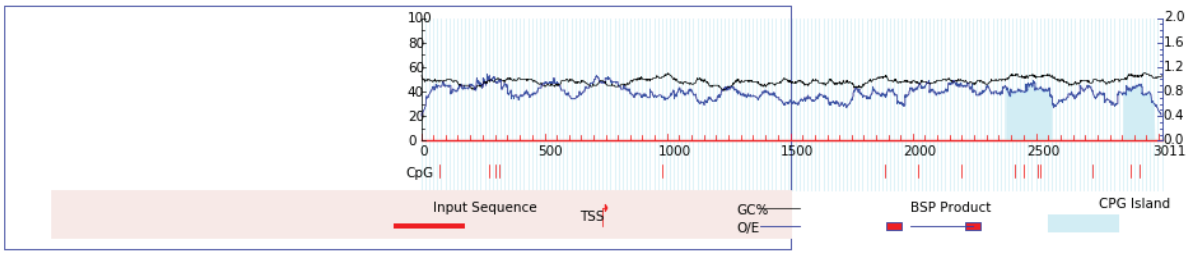

Figure 3. Prediction of methylation sites in the CpGi from Hepatitis C virus genotype 1
Source: study result.

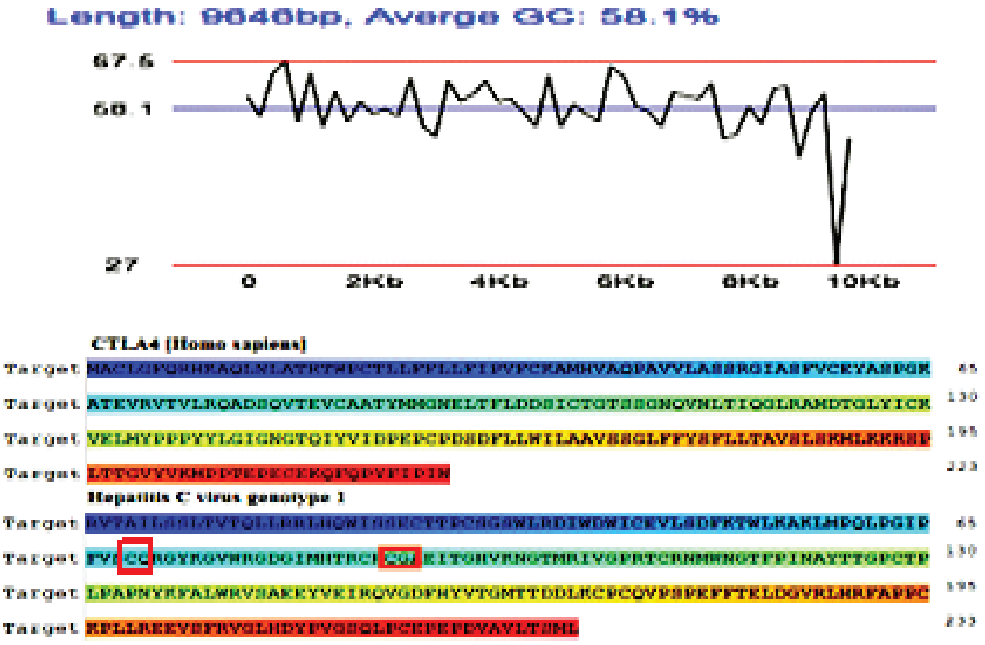

Figure 4. GC Content Distribution of HCV genotype 1
Source: study result.

Hepatitis C virus genotype 2, complete genome - CpGi Prediction

The parameters analysis and the primers for quantitative DNA methylation analysis of HCV genotype 2 Gene, and the sequence alignment of CTLA-4 and Hepatitis C genome revealed the presence of one putative promoters and one CpGi in the 3′ genomic region (**Figure 5 & 6**).

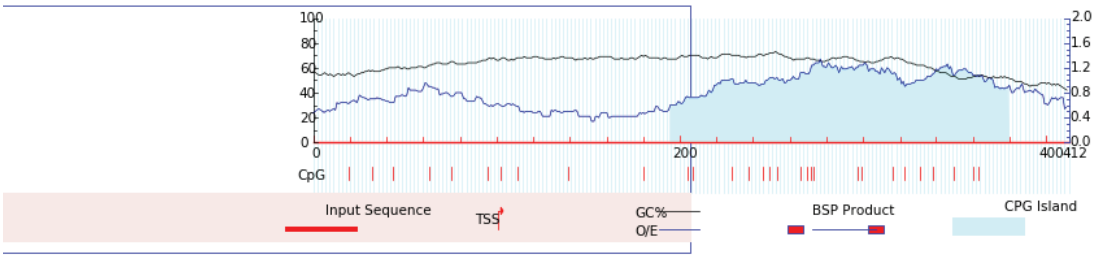

Figure 5. Prediction of methylation sites in the CpGi from HCV genotype 2
Source: study result.

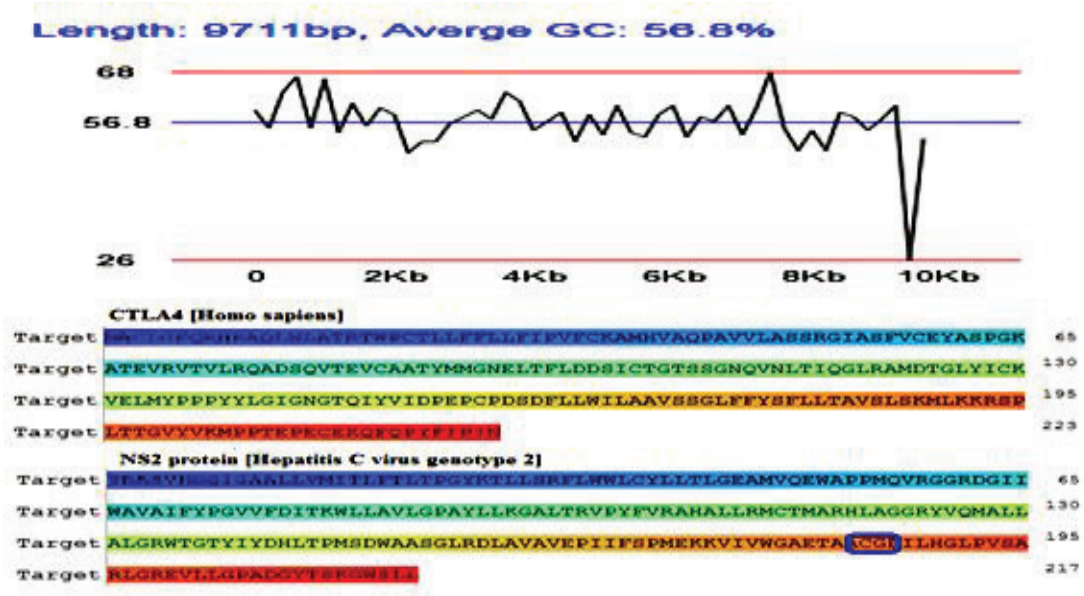

Figure 6. GC Content Distribution of HCV genotype 2
Source: study result.

Hepatitis C virus genotype 3, complete genome - CpGi Prediction

The in *silico* analysis and the primers for quantitative DNA methylation analysis of HCV genotype 3 Gene, and the sequence alignment of CTLA-4 and Hepatitis C genome revealed the presence of two putative promoters and two CpGi in the 5′ genomic region (**Figure 7 & 8**).

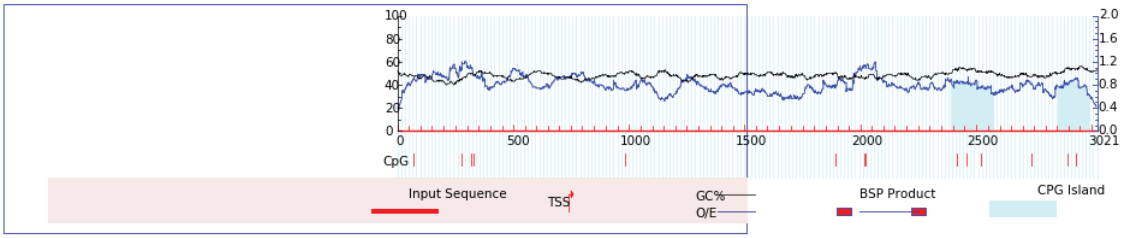

Figure 7. Prediction of methylation sites in the CpGi from HCV genotype 3
Source: study result.

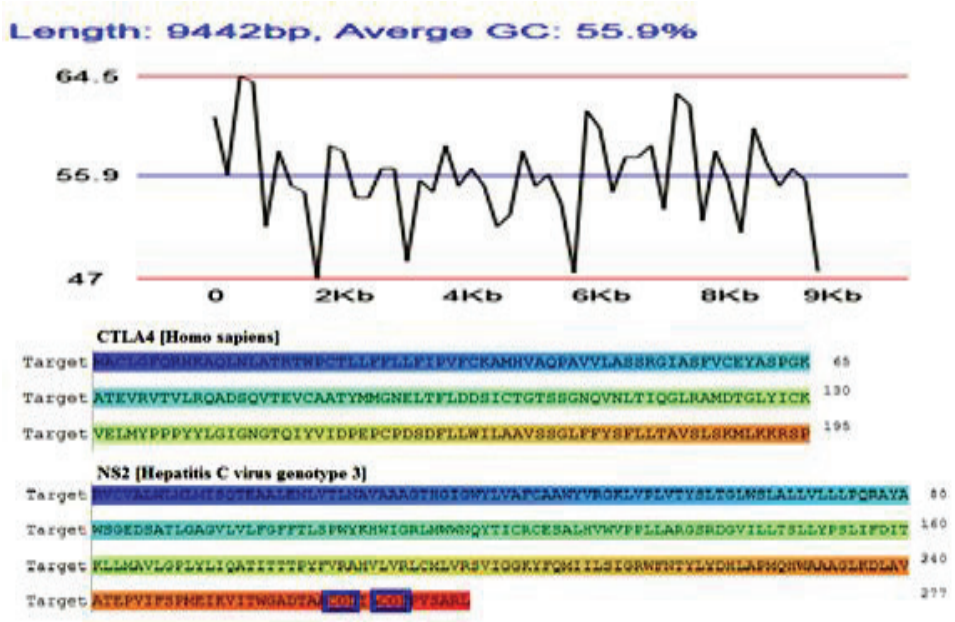

Figure 8. GC Content Distribution of HCV genotype 3
Source: study result.

To design of CTLA-4 primer was used Primer3 developed at NCBI using BLAST and global alignment algorithm to screen primers against selected database. The advanced and reverse primers were used to determine the methylation state of the CpGs within the CTLA-4 (**Figure 9**).

Primer Stats

Name	Considered	C's	G's	Target	Exclude	GC%	Gc_clamp	Tm_low	Tm_high	Comp_any	Comp_end	Poly_X	Poly_T	Stability	CG_low	CG_high	CG3	OK
Left	532	145	0	0	0	0	0	27	0	4	8	15	120	0	0	186	0	27
Right	1145	247	0	0	0	0	0	133	21	10	183	36	58	0	0	181	0	276

Pair Stats

Name	Considered	Size_lt	Size_gt	Target	Tm_diff	Tm_low	Tm_high	Comp_any	Comp_end	Island	CG's	OK
Pair	670	382	0	0	10	0	0	0	4	0	228	46

Figure 9. Primer nucleotide sequences of CTLA-4
Source: study result.

The DNA oligonucleotide sequence was altered by overlaying a C and G base on to A and T bases to accurately reflect the sequence of methylation in the CTLA-4 (**Figure 10**).

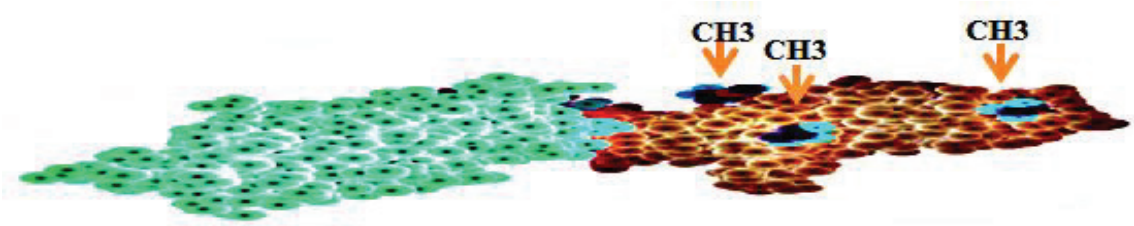

Figure 10. Methylation of CTLA-4
Source: study result.

DISCUSSION

Our *in silico* assessment suggests that epigenetic mechanisms may be underlying to triggering of autoimmune thyroiditis in individuals with HCV infection through of methylation of the CTLA-4 Gene promoter region.

Various extrahepatic disturbances are described associated to HCV infection, although the thyroids diseases be are generally the endocrinopathy most commonly diagnosed. The mechanisms of induced thyroid disease are complex but not entirely comprised (14).

Epigenetic disequilibrium promptly induces the progression of autoimmunity through regulation of immune cell functions (15). In diverse autoimmune diseases the epigenetic effect seems to perform a significant function in its triggering (16). Associated with this CTLA-4 that is essential immune checkpoint receptor regulating the T-cell activation, conferring predisposition to thyroid autoimmunity (17).

The human CTLA-4 Gene begins starting from 202 949•6 kb from the p-terminus of chromosome 2 and encompasses 6•2 kb on chromosome region 2q33 (18). NCBI based we present the location of structure of the CTLA-4, that has cytogenetic location in the 2q33.2, which is the long (q) arm of chromosome 2 at position 33.2, and molecular location in base pairs 203,867,771 to 203,873,965 on chromosome.

CpGi are regions with at GC frequency above 50%, at least 200 bp, and an observed-to-expected CpG ratio above 60%. CpGi describe potential CpGi regions employing the method reported by Gardiner-Garden and Frommer (19). We use the default parameters as proposed by Deaton and Bird (13) to identify the potential CpGi, and use as parameters length of DNA sequence > 300bp; CpG observed/CpG expected ratio > 60% and C+G% > 50%.

The cytosines in CpG dinucleotides are able to be methylated to form 5-methylcytosines, and circa of 70% to 80% of CpG cytosines are methylated in the mammals (20). In our analysis, the sequence alignment of CTLA-4 and HCV genome revealed the presence of two putative promoters and two CpGi in the 5′ genomic region of HCV genotype 1 Gene, one putative promoters and one CpGi in the 3′ genomic region of HCV genotype 2 Gene, and the presence of two putative promoters and two CpGi in the 5′ genomic region for HCV genotype 3 Gene. We did not find any published articles that associated the sequences alignment of CTLA-4 and HCV genotype 3 Gene, CTLA-4 and HCV genotype 2 Gene, and CTLA-4 and HCV genotype 1 Gene.

The Hashimoto's thyroiditis and Grave's disease are the most frequent autoimmune manifestations that occur in the thyroid (21). Recent studies demonstrated that DNA methylation is present in autoimmune thyroid disease determining a substantial epigenetic effect, being verified hypermethylated gene loci of CTLA-4 simultaneously to T cell receptor signaling (22). Our in silico analysis and the primers for quantitative DNA methylation analysis of thryroid peroxidase, and the sequence alignment of CTLA-4 and thyroid peroxidase not revealed the presence of putative promoters and none CpGi, but the advanced and reverse primers used to determine the methylation state of the CpGs within the CTLA-4 demonstrated that the DNA oligonucleotide sequence altered by overlaying a cytosine and guanine base on to adenine and thymine bases accurately reflected the sequence of methylation in the CTLA-4.

Recent studies proved that HCV infection as an important infectious triggering factor of autoimmune thyroiditis (23), and although the precise way of this association still is uncertain, one of potentials mechanism is related to epigenetic alterations as demonstrated in our study. Thus, epigenetics alterations have been to play a role important in the trigger of autoimmune thyroiditis.

CONCLUSION

The role of CTLA-4 methylation in the trigger of autoimmune thyroid disease in HCV infection was *in sílico* evaluated in the present study, and the results showed that CTLA-4 methylation can be a factor that induces the trigger of autoimmune thyroiditis. Nevertheless, in vitro and in vivo researches are needed to better clarify the exact implicit mechanism in deregulation of thyroid peroxidase activities in HCV infection by CTLA-4 methylation. We conclude that, in susceptible individuals, hypermethylation can promote reduced CTLA-4 expression and increases the risk of autoimmune thyroiditis in HCV-infected individuals.

References

1. Teft WA, Kirchhof MG, Madrenas J. A molecular perspective of CTLA-4 function. **Annu Rev Immunol**. 2006;24:65-97.
2. Lei C, Dongqing Z, Yeqing S, Oaks MK, Lishan C, Jianzhong J, et al. Association of the CTLA-4 gene with rheumatoid arthritis in Chinese Han population. **Eur J Hum Genet**. 2005;13(7):823-8.
3. Van Speybroeck L. From epigenesis to epigenetics: the case of C. H. Waddington. *Ann N Y Acad Sci*. 2002;981:61-81.
4. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. **Genes Dev**. 2009;23(7):781-3.
5. Suganuma T, Workman JL. Signals and combinatorial functions of histone modifications. **Annu Rev Biochem**. 2011;80:473-99.
6. D'Urso A, Brickner JH. Mechanisms of epigenetic memory. **Trends Genet**. 2014;30(6):230-6.
7. Wang F, Higgins JM. Histone modifications and mitosis: countermarks, landmarks, and bookmarks. **Trends Cell Biol**. 2013;23(4):175-84.
8. Brooks WH, Le Dantec C, Pers JO, Youinou P, Renaudineau Y. Epigenetics and autoimmunity. J Autoimmun. 2010;34(3):J207-19.
9. Lilley CE, Chaurushiya MS, Weitzman MD. Chromatin at the intersection of viral infection and DNA damage. **Biochim Biophys Acta**. 2010;1799(3-4):319-27.
10. Schott E, Witt H, Hinrichsen H, Neumann K, Weich V, Bergk A, et al. Gender-dependent association of CTLA4 polymorphisms with resolution of hepatitis C virus infection. **J Hepatol**. 2007;46 (3):372-80.
11. Wang B, Shao X, Song R, Xu D, Zhang JA. The Emerging Role of Epigenetics in Autoimmune Thyroid Diseases. **Front Immunol**. 2017;8: 396.
12. Methprimer. [(accessed on July 30, 2022)]. Available from: http://www.urogene.org/methprimer.
13. Deaton A, Bird A. CpG islands and the regulation of transcription. **Genes and Development.** 2011;25(10):1010–1022.
14. Andrade LJ, Atta AM, Atta ML, Mangabeira CN, Paraná R. Thyroid disorders in patients with chronic hepatitis C using interferon-alpha and ribavirin therapy. **Braz J Infect Dis**. 2011; 15 (4):377-81.
15. Moosavi A, Motevalizadeh AA. Role of epigenetics in biology and human diseases. **Iran Biomed J**. 2016;20(5):246–58
16. Hasham A, Tomer Y. Genetic and epigenetic mechanisms in thyroid autoimmunity. **Immunol Res**. 2012;54(1-3):204-13.
17. Chistiakov DA, Turakulov RI. CTLA-4 and its role in autoimmune thyroid disease. **J Mol Endocrinol**. 2003;31 (1):21-36.
18. Dariavach P, Mattéi MG, Golstein P, Lefranc MP. Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. **Eur J Immunol**. 1988;18 (12):1901-5.
19. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. **J Mol Biol**. 1987;196 (2):261-82.
20. Jabbari K, Bernardi G. Cytosine methylation and CpG, TpG (CpA) and TpA frequencies. **Gene**. 2004;333:143-9.
21. Lee HJ, Li CW, Hammerstad SS, Stefan M, Tomer Y. Immunogenetics of autoimmune thyroid diseases: A comprehensive review. **J Autoimmun**. 2015;64:82-90.
22. Cai TT, Muhali FS, Song RH, Qin Q, Wang X, Shi LF, et al. Genome-wide DNA methylation analysis in Graves' disease. **Genomics**. 2015;105(4):204-10.
23. Sousa Mde A, Paraná R, Andrade LJ. Sequence similarity between thyroid self-protein and hepatitis c virus polyprotein: possible triggering mechanism of autoimmune thyroiditis. **Arq Gastroenterol**. 2016;53 (3):185-91.

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PATIENT'S KNOWLEDGE IN RHEUMATOID ARTHRITIS: A STUDY OF ASSOCIATION WITH DISEASE ACTIVITY AND FUNCTIONALITY

UM ESTUDO ACERCA DA ASSOCIAÇÃO ENTRE ATIVIDADE DE DOENÇA, FUNCIONALIDADE E CONHECIMENTO DOS PACIENTES EM ARTRITE REUMATOIDE.

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Key words: Rheumatoid arthritis, Quality of life, Inflammation, Educational management..
Descritores: Artrite reumatoide. Qualidade de vida. Inflamação. Manejo educacional

Abstract

Introduction: Rheumatoid arthritis (RA) is an autoimmune disease characterized by the involvement of the synovial membrane mainly of peripheral joints. It is relevant to evaluate whether the patient's degree of knowledge about rheumatoid arthritis interferes with the function and control of the disease, and whether the patient's quality of life can be improved. **Aim:** To evaluate whether the degree of patient's knowledge about RA interferes with patient's function, control of the disease and quality of life. **Material and Methods:** Cross-sectional observational study of patients with an established diagnosis of RA. Epidemiological data, data on disease activity (measured by DAS or disease activity score 28 - ESR or erythrocyte sedimentation rate, DAS 28 PCR or C-reactive protein), function (measured by HAQ or Health Assessment Questionnaire) and quality of life (measured by the SF-12 or Short Form Health Survey with 12 questions) were collected. For the knowledge of the disease, the PKQ questionnaire (Patient's knowledge questionnaire) was applied. **Results:** About 101 patients were included. In general, the sample had very low knowledge about the disease with median PKQ of 14.0. It was not possible to demonstrate correlation between the level of patient's knowledge and disease activity (DAS-28 VHS, DAS 28 PCR), with HAQ or SF-12 (all with p>0.5). However, a positive correlation was found with the level of education. (P<0.0005; rho=0.33; 95% CI=0.14 to 0.50). **Conclusion:** This study demonstrated lack of knowledge about RA and its treatment by the patients. There was no correlation between the level of knowledge of the disease and variables of disease activity or cumulative damage. The number of years of formal study positively influenced the disease knowledge. **Endocrinol diabetes clin exp 2022 / 2341-2344.**

Resumo

Introdução: A artrite reumatoide (AR) é uma doença autoimune caracterizada pelo acometimento da membrana sinovial principalmente de articulações periféricas. É relevante avaliar a se o grau de conhecimento do paciente sobre artrite reumatoide interfere com a função e controle da doença, e ainda se pode melhorar a qualidade de vida do doente. **Objetivo:** Avaliar se o grau de conhecimento do paciente sobre artrite reumatoide interfere na sua função, controle da doença e qualidade de vida. **Material e Métodos:** Estudo transversal observacional de pacientes com diagnóstico estabelecido de artrite reumatoide. Coletaram-se dados epidemiológicos, de atividade de doença (medida pelo DAS ou disease activity score 28 - VHS ou velocidade de hemossedimentação e DAS 28 PCR ou proteína C reativa), de funcionalidade (medida pelo HAQ ou Health assessment questionnaire) e qualidade de vida (medida pelo SF-12 ou Short Form Health Survey with 12 questions) sendo aplicado para o conhecimento da doença, o questionário PKQ (Patient's knowledge questionnaire). **Resultados:** Incluíram-se 101 pacientes. A amostra de maneira geral tinha conhecimento muito baixo acerca da doença com PKQ mediano de 14,0. Não foi possível demonstrar correlação do nível de conhecimento sobre a doença com as medidas de atividade (DAS-28 VHS e DAS 28 PCR) nem com o HAQ ou SF-12 (todos com p>0.5). Todavia encontrou-se uma correlação positiva com o nível de escolaridade. (P<0.0005; rho=0,33;95% IC=0.14 a 0.50). **Conclusão:** Este estudo demonstrou a falta de conhecimento sobre a AR e de seu tratamento por parte dos pacientes. Não se encontrou correlação do nível de conhecimento da doença com variáveis de atividade ou de dano cumulativo da doença. A escolaridade influenciou de maneira positiva no conhecimento sobre a doença. **Endocrinol diabetes clin exp 2022 / 2341 - 2344.**

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that may cause injury to patient's joints leading to pain and deformities and impacting self-care and quality of life (1). It is a life changing disease. Patients' education is one of the first steps to be taken in the treatment of rheumatic disease. The patient's ability to understand the disease and how to deal with practical, physical and psychological impacts that come along with the disease is helpful in the self-management of difficulties that may appear as well as improves treatment adherence (2-4).

Nevertheless, this is an aspect that is frequently over-

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looked. Herein we studied a sample of RA patients aiming to identify the degree of knowledge in a local sample, its influence in the disease activity index and in the patients' functionality. It was hypothesized that a good understanding of diseases pathophysiology and consequences as well as on the measures used for joint protection will help avoiding joint deformities and in the treatment adherence resulting consequently in less disease activity and less musculoskeletal structural damage

MATERIAL AND METHODS

This is as cross-sectional study approved by local Committee of Ethics in Research. All participants sign consent. Males and females older than 18 years were recruited. To participate the patients should fulfill the classification criteria for Rheumatoid Arthritis from ACR (American College of Rheumatology/ EULAR (European League Against Rheumatism) 2010 (5).

Data collection included epidemiological, clinical, laboratorial and treatment data that were obtained through chart review. Function was measured by HAQ (Health Assessment Questionnaire), and the activity of disease was accessed through DAS 28 (disease activity score) – ESR (erythrocyte sedimentation rate) and DAS 28- CRP (C reactive protein). SF-12 (Short Form Health Survey with 12 questions) was applied for quality of life and the patient's knowledge on RA was assessed through the PKQ (Patients Knowledge Questionnaire). All used instrument were translated and validated to Portuguese language.

The HAQ is a questionnaire on daily activities that is scored from 0 to 3, where 0 means no impairment and 3 severe impairment of musculoskeletal function (6,7).

The DAS-28 is an index that takes into account the number of inflamed and sore joints of a pre-established map of 28 joints, ESR or CRP and a note given by the patient by visual analog scale about general health (where 0 means that the patient is well, without symptoms and 10 that is in the worst possible situation). A DAS-28 of up to 2.6 means that the patient is well-controlled AR. Above 2.6 to 3.2, activity considered mild, between 3.2 and 5.1 the activity is moderate and above that is high (8).

SF-12 measures health and mental quality of life through 12 questions; it ranges from 0 (worst result) to 100 (best results) (9).

The PKQ (10) analyzes several domains of patient's knowledge in the disease: (1) - about etiology and laboratorial tests rated from 0 to 10; (2) - about medications used for treatment rated from 0 to 6; (3) - about exercising rated from 0-6; (4) about joint protection and energy conservation rated from 0-8. The global score ranges from 0 (worst result) to 30 (best result).

Data was collected in frequency and contingency tables. Frequency of nominal data was expressed in percentages and numerical data in medians or media according to sample distribution. Correlation was studied by Spearman's test. The adopted significance was of 5%.

RESULTS

The sample had 101 RA patients. The epidemiological and clinical data is on Table 1 that shows predominance of middle-aged females with seropositivity and few extra articular manifestations. Methotrexate was the common drug used for treatment.

The results of disease activities measurements and applied questionnaires is on **Table 2**.

In this sample, the PKQ ranged from 3.0 to 21.0 (median of 14; IQR= 9.7-17.0). The patients' performance in the different domains of PKQ is on **Table 3,** showing median values under 50% in all but one domain (exercises).

Figure 1 shows the median values of each domain of PKQ expressed in % of the maximum possible score. It highlights that the domain less known was on drug treatment.

The correlation studies of PKQ values with inflammatory activities indexes and SF-12 is on **Table 4**. No correlations were found.

Also, no correlations of PKQ were found with HAQ (p=0.94), neither with disease duration (p=0.10) but a positive correlation was found with years of formal study (p=0.005; Rho=0.33 95%CI=0.14 to 0.50).

Table 1. Epidemiological, clinical and treatment profile of studied sample (101 rheumatoid arthritis patients)

Age (years)	Range – 27 to 82	Median 56 (48-62)
Female gender (n)	92/101-91.0%	
Age at diagnosis (years)	Range – 16 to 76	Mean 43.5±12.0
Years of formal study (n)	Range - 0 to 16	Median 8 (5-12)
Rheumatoid factor (n)	77/101 - 76.2%	
Rheumatoid nodules (n)	1/101 - 0.9%	
Scleritis (n)	2/101 - 1.9%	
Interstitial lung disease (n)	1/101 - 0.9%	
Comorbidities		
Arterial hypertension (n)	39/101 – 38.6%	
Dyslipidemia (n)	51/101 – 50.,4%	
Diabetes mellitus (n)	14/101 – 13.8%	
Depression (n)	16/101 – 15.8%	
Treatment		
Antimalarials (n)	11/101 – 10.8%	
Methotrexate (n)	53/101 – 52.4%	
Leflunomide (n)	48/101 – 47.8%	
Glucocorticoid (n)	41/101 – 40.5%	
Anti TNF-alpha (n)	27/101 – 26.7%	
Anti-IL6 (n)	11/101 – 10.8%	
Abatacept (n)	7/101 – 6.9%	
Rituximab (n)	7/101 – 6.9%	
Tofacitinib (n)	5/101 – 4.9%	

Table 2. Inflammatory activity indicators, functional index and quality of life in the studied sample (101 patients with Rheumatoid Arthritis)

	Median (interquartile range)
DAS 28 ESR	3.07 (2.28-4.17)
DAS 28 CRP	2.40 (1.55-3.42)
CRP (mg/dL)	2.9 (0.8-8.8)
ESR (mm)	22.5 (11.5- 44.0)
HAQ	1.0 (0.5- 1.5)
SF-12 mental domain	43.7 (33.6-52.2)
SF-12 physical domain	36.9 (28.9-42.0)

DAS- disease activity score; CRP= C reactive protein; ESR= erythrocyte sedimentation rate; HAQ= health assessment questionnaire, SF-12 = Short Form Health Survey with 12 questions

Table 3. Obtained values in the PKQ by 101 rheumatoid arthritis patients

Domain	Highest possible value	Noemal Range	Median (IQR)
Etiology/laboratorial exams	10	0-8	4 (3-6)
Drug treatment	6	0-5	2 (2-3)
Exercises	6	0-6	4 (3-4.5)
Joint protection and energy conservation	8	0-6	3 (2-4)

PKQ= patients' knowledge questionnaire; IQR= interquartile range.

Figure 1. Domains of PKQ (patient's knowledge questionnaire) values expressed in % of maximum possible score in 101 patients with Rheumatoid arthritis

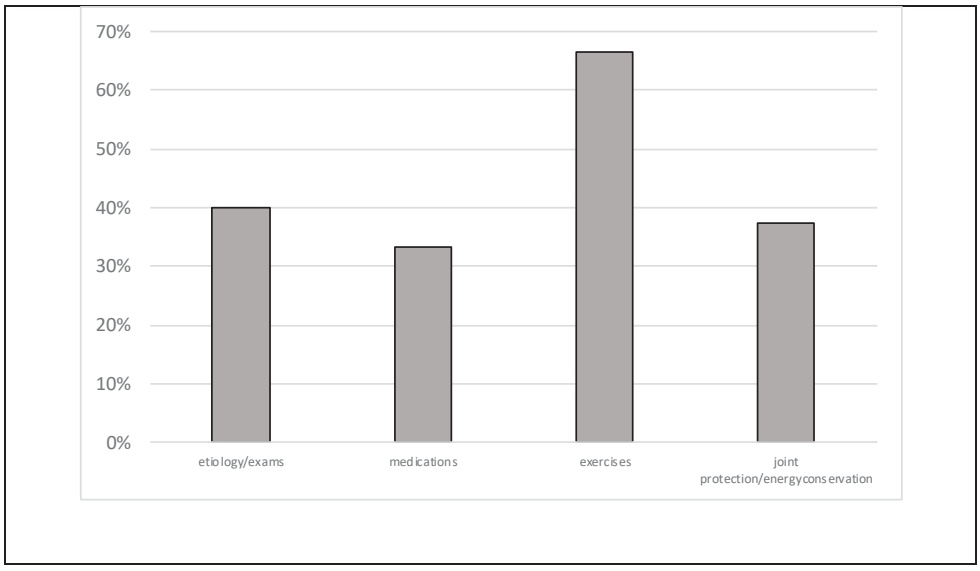

Table 4. Correlation studies of PKQ (patients' knowledge questionnaire) with inflammatory indexes, and quality of life in 101 rheumatoid arthritis patients

	Spearman rho	95% confidence interval	P
DAS 28 ESR	0.10	-0.09 to 0.29	0.29
DAS 28 CRP	-0.05	-0,24 to 0.15	0.61
SF-12 physical domain	-0.09	-0.28 to 0.11	0.36
SF-12 mental domain	0.02	-0,17 to 0.22	0.77

DAS-28 = disease activity score;ESR= erythrocyte sedimentation rate; CRP= C reactive protein; SF-12=Short Form Health Survey with 12 questions.

DISCUSSION

Our results have failed to show association of patients' knowledge on RA with disease activity, patients function or quality of life. The most striking finding was the very low level of patients' knowledge in all studied domains pointing for an urgent need to educate the RA patient.

Abourazzak et al. (11), contrarily from the present results, have found that an educational program of 3 days done in a sample of 39 patients helped in disease control until 3 years from baseline, but they failed to associated it with patient's functionality measured by HAQ. The study by Giraudet-Le Quintrec et al. (12) with 208 patients submitted to a randomized controlled study did not show association of an educational program with HAQ scores after one year but patient coping, knowledge, and satisfaction were improved in the intervention group. A literature review in therapeutic patients' education (13) has shown that most of studies show short term beneficial effects on the basis of bio-clinical, educational, psychosocial and economic criteria. However, these aspects were not studied presently.

The lack of association between knowledge of the disease and its activity in the present study may be due to the effort of the attending rheumatologist to use the treat to target therapy - in which all efforts are made to decrease inflammatory activity, even when it is small. This may have obliterated the importance of the patient's knowledge in this context.

Even though the direct effect of education on disease activity may not have been proven, the knowledge gains documented in Abourazzak's study (11) and the results previously published in other studies suggest that patient education programs may increase treatment regimen effect through a better understanding of their importance and better acceptance of their side effects.

CONCLUSION

Concluding, the present study showed that local RA patients have a very low knowledge on their disease. It was not possible to prove association of patients' knowledge with disease inflammatory control neither with patient's functionality.

References

1- Ngian GS. Rheumatoid arthritis. **Aust Fam Physician**. 2010; 39 (9):626-8.

2- Marques A, Santos E, Nikiphorou E, Bosworth A, Carmona L. Effectiveness of self-management interventions in inflammatory arthritis: a systematic review informing the 2021 EULAR recommendations for the implementation of self-management strategies in patients with inflammatory arthritis. **RMD Open**. 2021:7 (2):e001647.

3- Fayet F, Pereira B, Fan A, Rodere M, Savel C, Berland P, et al. Therapeutic education improves rheumatoid arthritis patients' knowledge about methotrexate: a single center retrospective study. **Rheumatol Int**. 2021 May 29. doi: 10.1007/s00296-021-04893-5. On line ahead of print.

4- Nikiphorou E, Santos EJF, Marques A, Böhm P, Bijlsma JW, Daien CI, et al. 2021 EULAR recommendations for the implementation of self-management strategies in patients with inflammatory arthritis. **Ann Rheum Dis**. 2021 May 7: annrheumdis-2021-220249. doi: 10.1136/annrheumdis-2021-220249. Online ahead of print.

5- Goeldner I; Skare TL; Reason IM, Utiyama SR. Artrite reumatoide: uma visão atual. **J. Bras. Patol. Med. Lab**. 2011;47 (5): 495-503.

6- Bruce B, Fries JF. The Health Assessment Questionnaire (HAQ). **Clin Exp Rheumatol**. 2005 Sep-Oct; 23(5 Suppl 39):S14-8.

7- Orlandi AC, Cardoso FP, Santos LM, Cruz Vda G, Jones A, Kyser C, Natour J. Translation and cross-cultural adaptation of the Scleroderma Health Assessment Questionnaire to Brazilian Portuguese. **São Paulo Med J**. 2014;132(3):163-9.

8- Medeiros MM, de Oliveira BM, de Cerqueira JV, Quixadá RT, de Oliveira ÍM. Correlation of rheumatoid arthritis activity indexes (Disease Activity Score 28 measured with ESR and CRP, Simplified Disease Activity Index and Clinical Disease Activity Index) and agreement of disease activity states with various cut-off points in a Northeastern Brazilian population. **Rev Bras Reumatol**. 2015; 55(6): 477-84.

9- Silveira MF, Almeida JC, Freire RS, Haikal DS, Martins AE de BL. Propriedades psicométricas do instrumento de avaliação da qualidade de vida: 12-item health survey (SF-12) 12-item health survey (SF-12). **Cien Saude Colet**. 2013;18 (7):1923–31

10- Jennings F, Toffolo S, de Assis MR, Natour J. Brazil Patient Knowledge Questionnaire (PKQ) and evaluation of disease-specific knowledge in patients with rheumatoid arthritis. **Clin Exp Rheumatol**. 2006 Sep-Oct; 24 (5):521-8.

11- Abourazzak F, El Mansouri L, Huchet D, Lozac'hmeur R, Hajjaj-Hassouni N, Ingels A, et al. Long-term effects of therapeutic education for patients with rheumatoid arthritis. **Joint Bone Spine**. 2009; 76(6): 648-53

12- Giraudet-Le Quintrec JS, Mayoux-Benhamou A, Ravaud P, Champion K, Dernis E, Zerkak D, et al. Effect of a collective educational program for patients with rheumatoid arthritis: a prospective 12-month randomized controlled trial. **J Rheumatol**. 2007; 34(8):1684-91

13- Albano MG, Giraudet-Le Quintrec JS, Crozet C, d'Ivernois JF. Characteristics and development of therapeutic patient education in rheumatoid arthritis: analysis of the 2003-2008 literature. **Joint Bone Spine**. 2010 ;77(5):405-10

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TOPICS IN CLINICAL MEDICINE

ORIGINAL ARTICLE
DRY EYE AND ACITRETIN: A TRANSVERSAL STUDY AND A SYSTEMATIC REVIEW OF THE LITERATURE

USO DE ACITRETINA E OLHO SECO: UM ESTUDO TRANSVERSAL E REVISÃO SISTEMÁTICA DA LITERATURA.

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THELMA L SKARE [2]

Key-words: Dry eye syndrome; Meibomian glands; 13 cis-retinoic acid.
Descritores: Síndrome do Olho seco; Glândulas de Meibomio; Ácido 13 cis-retinoico

Abstract

Background: Isotretinoin is used for treatment of acne and such medication have mucocutaneous side effects; this drug has influence in functionality of the sebaceous and meibomian glands. The impairment of meibomian glands can lead to disruption of the tear film and consequent symptoms of evaporative dry eye. It is believed that dry eye syndrome is associated with the mechanism of action of the isotretinoin. **Objectives:** Verify the prevalence of dry eye in a series of patients using isotretinoin and review systematically the literature of the past 10 years about this subject. **Material and Methods:** Study of a series of patients in use of isotretinoin: it includes patients of both genders, from Dermatology Outpatient Clinic of Hospital Evangélico Mackenzie do Paraná, using isotretinoin. We collected epidemiologic and treatment data. The patients were subjected to the tBUT to test tear film stability. The results were compared with a gender- and age- matched population not using this drug and without acne. Three databases were reviewed utilizing the term "Isotretinoin and Dry Eye" and "Acitretin and Dry Eye" on the past 10 years. We obtained a total of 126 titles. After applying exclusion and inclusion criteria we selected 7 articles that were reviewed in full which were systematically summarized. **Results:** Study of the series of patients using isotretinoin: The average time in which the lacrimal film rupture occurred was 7.2 seconds on patients using isotretinoin and 9.4 seconds on control patients with p=0.01. Age and treatment duration did not interfere with the results. **Literature Review:** 5 of the 7 selected articles were prospective, 1 retrospective and 1 experimental. The literature findings corroborate the decrease in time of the tBUT on those using isotretinoin, causing dry eye and point to Meibomian gland disfunction as the leading cause. **Conclusion:** Patients using isotretinoin have decreased time in tear film rupture time due to the use of isotretinoin. This is caused by the meibomian gland disfunction associated with dry eye. **Endocrinol diabetes clin exp 2022 / 2349 - 2221.**

Resumo

Introdução: A isotretinoína é usada para tratamento da acne e tal medicamento tem efeitos adversos muco-cutâneos; influencia a funcionalidade das glândulas sebáceas e também a das glândulas meibomianas. A afecção das glândulas de Meibomio pode levar a desestabilização da lágrima e aparecimento de olho seco evaporativo. Assim acredita-se que a síndrome do olho seco está associada ao mecanismo de ação da Isotretinoína. **Objetivos:** Verificar a prevalência de olho seco em uma série de usuários de isotretinoína e rever sistematicamente a literatura dos últimos 10 anos sobre este assunto. **Material e Métodos:** Estudo da série de pacientes em uso de isotretinoína: Foram incluídos pacientes de ambos os sexos do ambulatório de dermatologia do Hospital Evangélico Mackenzie do Paraná, em uso de isotretinoína. Coletaram-se dados epidemiológicos e de tratamento da acne e o paciente foi submetido ao exame do tBUT (Break up time ou tempo de ruptura lacrimal) para análise de estabilidade lacrimal. Os resultados foram comparados com população pareada para sexo e idade não usuária deste medicamento e sem acne. **Revisão de literatura:** Três bases de dados foram revisadas para literatura dos últimos 10 anos usando-se os termos "Isotretinoin and Dry eye" e "Acitretina and Dry Eye". Obteve-se um total de 126 títulos. Após aplicados critérios de inclusão e exclusão selecionaram-se 7 artigos que foram revisados na íntegra os quais foram resumidos sistematicamente. **Resultados:** Estudo da série de pacientes em uso de isotretinoína: O tempo médio de quebra da lágrima em usuários de isotretinoína foi de 7.2 seg. e dos controles de 9.4 seg. com p=0.01. Idade e duração do tratamento não influíram nos resultados. **Revisão de literatura:** Dos 7 artigos selecionados 5 eram prospectivos, 1 retrospectivo e 1 experimental. Os achados de literatura corroboram a diminuição do tBut em usuários de isotretinoína causando olho seco e apontam para a disfunção meibomiana como a sua principal causa. **Conclusão:** Pacientes em uso de isotretinoína apresentam menor tempo de quebra da lágrima devido ao uso do medicamento. Isto é causado por disfunção de glândulas de Meibomio e se associam a olho seco. **Endocrinol diabetes clin exp 2022 / 2345 - 2349.**

INTRODUCTION

Dry eye results from tear dysfunction and may impair the integrity of ocular surface favoring corneal lesions and ulcers (1). It may result from diminished tear production from the lacrimal glands or from excessive tear evaporation due to Meibomian glands dysfunction. The Meibomian glands are responsible for producing an external lipidic layer that protects the tear from excessive evaporation (1).

Acitretin or 13-cis retinoic acid is a vitamin A analogous used for treatment of severe acne, psoriasis and other diseases with keratinization defects (2). This drug is a powerful inhibitor of sebaceous gland function and has anti-inflammatory and immunoregulatory functions. Midst its side effects, the mucocutaneous are some of most common; among them is the

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evaporative dry eye (3).

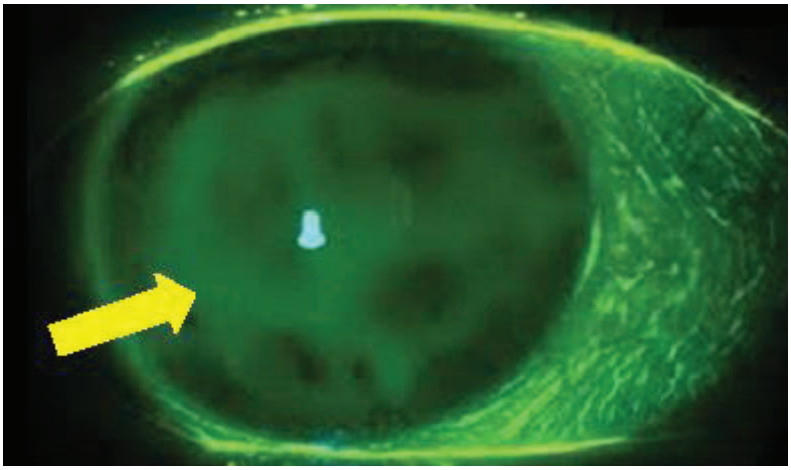
Herein we studied a sample if individuals using acitretin for acne treatment aiming to know the prevalence of dry eye and reviewed the literature from the last 10 years on this issue, summarizing the main findings.

MATERIAL AND METHODS

1. Original Study – This study was approved by the local committee of ethics in research and all participants sign a consent. To be included patients should be older than 18 years of age and to be using acitretin for acne treatment. The included patients were from both genders, from a single Dermatology Unit that came for regular consultation during the period of 1 year.

Data collection included: sex, age, treatment duration and acitretin dose. All of them had the tBUT (tear break up time) test done. The tBUT test is done with instillation of fluorescein tear drop in the conjunctival sac. After blinking, the patients are examined under the cobalt blue light of a slit-lamp for the time of appearance of the first dry spot (4), **Figure 1**. The normal value is of 10 seconds. As controls we included patients companion and people from the hospital staff. This is a test that is used to test the tear stability.

Figure1. Measurement of tear instability usin TBUT (break up time)



A fluorescein tear drop is instilled in the conjunctival sac. After blinking, the patient is examined under the cobalt blue light of a slit-lamp measuring the time for the appearance of the first dry spot (arrow). Normal=10 seconds

Obtained results were compared using the chi-squared test for nominal data and the unpaired t test for numerical variables. Correlation studies of tBUt with patients age and duration of treatment were done by Spearman test. The adopted significance was of 5%.

2. Literature review: The literature on dry eye and acitretin use from the last 10 years was systematically reviewed using the following Key words: dry eye AND acitretin and dry eye AND isotretinoin in three data bases Medline/ Pubmed, Embase and Scielo. The search was conducted by two individuals (NMP and TLS) and included original articles on adult humans and animals. Review articles, editorials and researches in humans under 18 years of age were excluded. The search was limited to the last 10 years (from 2012- 2022) and to papers in English, Portuguese and Spanish.

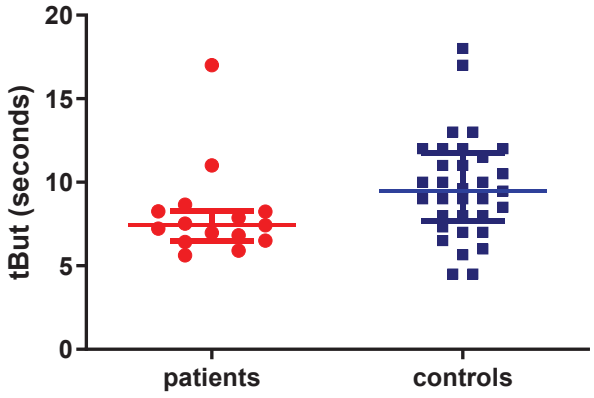
RESULTS

1. Original Study: Forty eight individuals were included: 18 using isotretinoin and 30 controls. In the isotretinoin users' sample 53.3% were males with median age of 23.0 (IQR= 20-24 years). In the control group 48.4% were males with median age of 23 years (IQR=22-32 years). Patients and controls were paired for age (p= 0.10) and gender (p=1,0). The use of isotretinoin ranged from 1 to 12 months (median of 6; IQR=3-12 months). The comparison of tBUT results is on **figure 1**, that shows that the median values of tBUT was lower in those using isotretinoin.

Correlation studies of tBUT value with patients' age and treatment duration were not significant (p=0.41 and 0.72 respectively).

The abstract of studied articles is on **Table 1**. It included 7 original papers: 5 prospective studies, 1 retrospective study and 1 experimental (5-11).

Figure 1. Comparison of tBUT results in patients using isotretinoin for acne treatment and controls



Isotretinoin sample: median value of 7.2 sec. (IQR=6.5-8.2 sec.); Controls: median value of 9.4 sec. (IQR=7.6=11.7 sec); p=0.01

2. Literature review: The flowchart of articles selection is on **figure 2**

Figure. Flow chart of systematic review on dry eye and isotretinoin

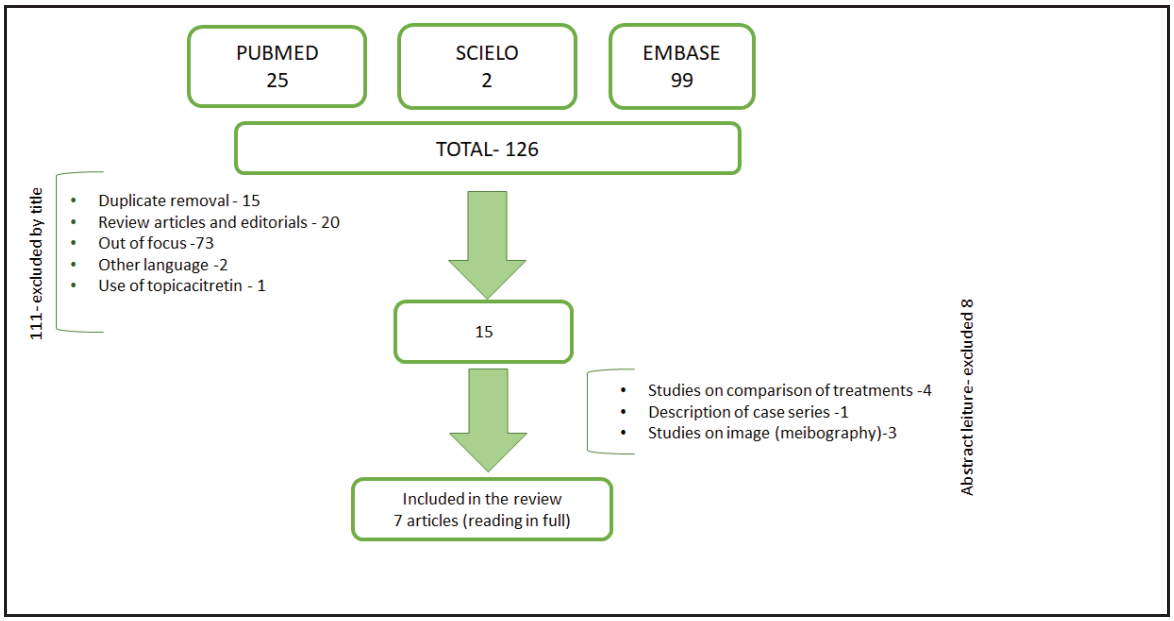


Table 1. Studies on dry eye and Isotretinoin use for acne treatment (2012 to 2022)

Author/Year	N (eyes/patietns)	Study design	Used evaluation instruments	Findings	Conclusion
Gurlevik et al, 2022 (5).	30 eyes/30 patients	Prospective (1,3,6 months after treatment)	Fluorescein, tBUT, Schirmer test, meibography	Loss of Meibomian glands in upper eyelid – 22 % (3 months) and 23.6% (6 months). Lower eyelid – loss of 18.7% (3 months) and 20.7% (6 months)	Isotretinoin changes meibomian gland morphology and causes evaporative dry eye.
Caglar et al, 2016 (6)	120 eyes /60 pacients	Prospective	tBUT, Schirmer test, OSDI, biomicroscopic examination of meibomian gland	Schirmer scorer did not change but OSDI and tBUT had significant changes	Systemic use of isotretinoin causes alterations in the tear composition and symptoms of dry eye.
Tanrivedi et al, 2021 (7).	88 eyes/88 pacients	Prospective (0, 4 and 8 months)	Meibography without contact, tBUT non-invasive, tBUT invasive, OSDI and MGD	tBUT values lowered during isotretinoin use: Mean of- 13.78sec. (0 months), 11.49sec. (1 month), 9.92sec. (3 months), 10.00 sec. (six months). MGD showed mean value of 0.29±0.45 before treatment and 0.97±0.87 after treatment.	Systemic use of isotretinoin causes morphological changes in MG and may affect negatively the lacrimal film. This finding was present 12 months after the treatment was finished.
Brzezinski et al, 2017 (8).	3525 patients	Retrospective (4 years) from two populations: Polish and Romanian	Chart study. Observation of 40 side effects (including dry eye).	Less than 10% of patients had dry eye (n=201). The most common were: mucocutaneous that appeared in 100%.	The ocular side-effects of isotretinoin are moderate and well tolerated appearing in a minority of patients.

Author/year	N (eyes/patients)	Study design	Used evaluation instruments	Findings	Conclusions
Ding et al., 2013 (9).	*In vitro* -	Experimental. Exposition of immortalized MG epithelial cells to 13-cis retinoic acid.	Gene expression., cellular proliferation, apoptose degree, measurement of IL-1β, MMP-9 and phospho AKt.	Altered expression. of 6736 genes that were associated with MG cell proliferation, differentiation and cellular death. Reduction in Akt phosphorylation and ↑ of IL-1 and MMP-9.	13-cis retinoic acid causes alterations in gene expression. and an increase in apoptose and inflammatory mediators in MG cells culture
Düzgün & Özkur, 2020 (10).	45 eyes/ 45 patients	Prospective (0 and 4 months)	tBUT, OSDI, fluorescein, and Schirmer. Meibography. Observation of abnormalities in the eyelids.	tBUT mean time ↓ of 8.9 sec. to 7.6 sec after 4 months of treatment. Changes in meibography scores in. 91.1% of upper lids and 37.7% of lower lids. Changes in density and size of MG.	Meibography without contact is useful to evaluate changes in MG related to isotretinoin use. Isotretinoin use ↓ number and size of MG.
Fouladgar et al, 2018 (11).	50 patients/	Prospective	Mesurement of corneal sensitivity by corneal aesthesiometry .	Corneal sensitivity ↓ from 5.54 ± 0.05 mm to 5.41 ± 0.05 mm after 3 months of treatment.	Corneal sensitivity ↓ with isotretinoin use for three months. This finding is more striking in women and elderly.

OSDI=ocular surface disease index; tBUT= breack up time MGD= Meibomian gland dysfunction .MG= Meibomian gland

DISCUSSION

The results of the tBUT analysis in the presently studied sample showed that there was a decrease in the time of tear rupture caused by a tear film instability in users of systemic isotretinoin when compared with controls. The tBUt test was considered the most indicated in this context since it analyzes the quality of the tear film and its ability to maintain adequate protection to the underlying tissues. This quality is dependent on the formation of a good lipid layer that prevents evaporation of the aqueous part of the tear (1). It is the Meibomian glands that have the important function of contributing to the homeostasis of the tear film producing this oily film that helps to prevent the evaporation of the tear film (1). In the evaporative dry eye, the tear film becomes unstable or deficient due to an inability to maintain the aqueous layer of the tear film (1,3). Consequently, the tear becomes hyperosmolar which triggers local inflammation. Inflammation can further damage the glands of Meibomian and lead to a vicious cycle of inflammation and refractory treatment disease (1).

The present findings are in agreement with the articles selected for the systematic review suggesting the occurrence of evaporative dry eye due to dysfunction and reduction in size and number of the Meibomian glands. The studies by Gurlevik et al., (2022) (5), Caglar et al. (2016) (6), Tanriverdi et al. (2021) (7) and Düzgün & Özkur (2020) (8) are illustrative in this context.

In a prospective study conducted by Gurlevik et al. (5), it was found that the systemic use of isotretinoin affects the morphology of the Meibomian gland as well as its density. These authors showed loss of meibomian glands of the upper eyelid lip of 22% in 3 months with increased loss to 23.6% at 6 months, and of the lower lip 18.7% in 3 months with increased loss to 20.7% in 6 months, suggesting that the process increases with the course of treatment. Similarly, Tanriverdi et al., (7) showed that systemic treatment with isotretinoin causes morphological changes in the Meibomian glands, besides negatively affecting the tear film of patients, with a decrease in tear rupture time from 13.78s to 10.00s in just 6 months.

However, Meibomian gland dysfunction is not the only given explanation for the pathophysiology of the dry eye associated with isotretinoin. The study by Fouladgar et al. (11) showed a second mechanism: the decrease in corneal sensitivity whose reflex is important for tearing, or for increasing the aqueous tear layer. This reflex is important for protection of the cornea, removing debris that may accumulate locally.

Still a third explanation for the dry eye in isotretinoin comes from the experimental study made by Ding et al. (9). It showed that 13-cis retinoic acid alters the expression of 6726 genes from the epithelial cells of the Meibomian glands - including those involved in cell proliferation, cell death, cell differentiation, keratinization and inflammation. In this study there was an increase in pro-inflammatory cytokines such as interleukin-1β and metallopeptidase matrix 9 (MMP-9) which increase the inflammatory process in the Meibomian glands. From this study it was concluded that 13-cis retinoic acid promotes effects that are partly related to the induction of the inflammatory process with consequent dysfunction of the meibomian gland.

The high sample number of patients by Brzezinski et al. (8) showed that dry eye is one of the less common side effects when using isotretinoin. Mucocutaneous complaints are much more frequent. This may contribute to the health professional not paying much attention to this type of side effect. However, it is necessary to emphasize that the recognition of the dry eye is important because it causes the symptoms of itching and local pain, irritation and red eye, and may favor lesions in the cornea promoting the appearance of corneal ulcers.

There are no long-term prospective studies on the repercussions of isotretinoin on the eye. However, the study by Tanriverdi et al. (7) found that these changes are still present up to 1 year after the end of treatment.

The authors from the recent review also observed that the literature of the last 10 years showed that there are not many studies on the subject, that deserves more research.

CONCLUSION

Isotretinoin users have lower tBut values when compared to those who do not use the drug, which increases the occurrence of dry eye. The systematic literature review corroborates the finding of decreased tBUt in the presence of isotretinoin use and points to the reduction of number of Meibomian gland as well as its dysfunction as the main cause.

References

1- Clayton JA. Dry Eye. **N Engl J Med**. 2018;378 (23):2212-2223. doi: 10.1056/NEJMra1407936.

2- Bagatin E, Costa CS. The use of isotretinoin for acne - an update on optimal dosing, surveillance, and adverse effects. E**xpert Rev Clin Pharmacol.** 2020; 13(8):885-897. doi: 10.1080/17512433.2020.1796637.

3- Brito M de F, Sant'Anna IP, Galindo JC, Rosendo LH, Santos JB. Evaluation of clinical adverse effects and laboratory alterations in patients with acne vulgaris treated with oral isotretinoin. **An Bras Dermatol**. 2010;85 (3):331-7. doi: 10.1590/s0365-05962010000300006.

4- Ayaki M, Negishi K. Decrease of tear break-up time at Japanese

eye clinics during five consecutive years. **Sci Rep**. 2022;12 (1):6848. doi: 10.1038/s41598-022-11035-9.
5- Gurlevik U, Kemeriz F, Yasar E. The effect of isotretinoin on meibomian glands in eyes: a pilot study. **Int Ophthalmol**. 2022 Jan 3. doi: 10.1007/s10792-021-02205-1.
6- Caglar C, Senel E, Sabancilar E, Durmus M. Reduced ocular surface disease index (OSDI) scores in patients with isotretinoin treatment. **Int Ophthalmol**. 2017 Feb; 37 (1):197-202. doi: 10.1007/s10792-016-0263-y.
7- Tanriverdi C, Nurozler Tabakci B, Donmez S. Longitudinal assessment of meibomian glands and tear film layer in systemic isotretinoin treatment. **Eur J Ophthalmol**. 2021; 20:11206721211018361. doi: 10.1177/11206721211018361.
8- Brzezinski P, Borowska K, Chiriac A, Smigielski J. Adverse effects of isotretinoin: A large, retrospective review. **Dermatol Ther**. 2017;30 (4). doi: 10.1111/dth.12483.
9- Ding J, Kam WR, Dieckow J, Sullivan DA. The influence of 13-cis retinoic acid on human meibomian gland epithelial cells. **Invest Ophthalmol Vis Sci**. 2013; 54(6):4341-50. doi: 10.1167/iovs.13-11863.
10- Düzgün E, Özkur E The effect of oral isotretinoin therapy on meibomian gland morphology and dry eye tests. **J Dermatolog Treat.** 2022 ;33(2):762-768. doi: 10.1080/09546634.2020.1774041.
11- Fouladgar N, Khabazkhoob M, Hanifnia AR, Yekta A, Mirzajani A. Evaluation of the effects of isotretinoin for treatment of acne on corneal sensitivity. **J Curr Ophthalmol.** 2018;30 (4):326-329. doi: 10.1016/j.joco.2018.06.005. eCollection 2018.

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CASE REPORT

THYROTOXIC HYPOKALEMIC PERIODIC PARALYSIS: CASE REPORT

PARALISIA PERIÓDICA HIPOCALÊMICA TIREOTÓXICA: RELATO DE CASO

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Key words: Muscle paralysis, Hyperthyroidism, Hypokalemia
Descritores: Paralisia muscular, Hipertiroidismo, Hipocalemia

Abstract

Thyrotoxic hypokalemic periodic paralysis is composed by the triad of muscle paralysis, thyrotoxicosis and acute hypokalemia without total body potassium deficit. This pathology is related to clinical or subclinical hyperthyroidism. The largest number of cases occurs in Asian patients, being uncommon in whites and blacks. It affects young adults between 20 and 40 years old, with men 20 times more affectionate than women. Other metabolic alterations that can be observed in patients with thyrotoxic hypokalemic periodic paralysis are hypokalemia, hypophosphatemia and mild hypomagnesemia. Emergency treatment consists of hypokalemia correction and control of thyrotoxicosis. **Endocrinol diabetes clin exp 2022 / 2350 - 2354.**

Resumo

A Paralisia periódica hipocalêmica tireotóxica é composta pela tríade paralisia muscular, tireotoxicose e hipocalemia aguda sem déficit total de potássio corporal. Essa patologia está relacionada ao hipertireoidismo clínico ou subclínico. O maior número de casos ocorre em pacientes asiáticos, sendo incomum em brancos e negros. Acomete adultos jovens entre 20 a 40 anos, sendo os homens afetados 20 vezes mais em relação às mulheres. Outras alterações metabólicas que podem ser observadas no paciente com paralisia periódica hipocalêmica tireotóxica são hipocalemia, hipofosfatemia e leve hipomagnesemia. O tratamento na emergência consiste na correção de hipocalemia e controle da tireotoxicose. **Endocrinol diabetes clin exp 2022 / 2350 - 2354.**

INTRODUCTION

Thyrotoxic hypokalemic periodic paralysis (THPP) is characterized by self-limited and recurrent episodes composed of the following triad: muscle paralysis, thyrotoxicosis and acute hypokalemia without total body potassium deficit (1).

It is a usually debilitating complication that can be life threatening related to clinical or subclinical hyperthyroidism. The largest number of cases occurs in Asian patients, being uncommon in white and black people. It affects young adults between 20 and 40 years old, with men being affected 20 times more than women. Metabolic alterations that can be observed in patients with THPP are hypokalemia, hypophosphatemia and mild hypomagnesemia (2).

The pathogenesis is still not very clear, but it is related to the hypokalemia that occurs as a result of intracellular translocation of K + due to greater activation of the Na+-K+ATPase pump as consequence of thyrotoxicosis. Hyperthyroidism may increase stimulation of pump activity by β2-adrenergic agonists, amplifying intracellular cAMP production. However, only 2% of patients with hyperthyroidism have THPP. Thus, activation of Na+–K+ ATPase should not be the only mechanism of the alteration (3).

The diagnostic suspicion occurs with the clinical manifestation of acute paralysis, related to precipitating factors, such as strenuous exercises and excessive consumption of carbohydrates and poorly controlled hyperthyroidism. Elevated thyroid hormones, thyroid-stimulating hormone suppression, and low serum potassium levels confirms the diagnosis. As the disease can be fatal, as soon as the suspicion is confirmed, drug therapy and patient monitoring should be started. Emergency treatment consists of correcting hypokalemia and controlling thyrotoxicosis (4). When serum potassium levels are normalized, normal muscle function is restored (2) (3).

We report a case of a male patient, of Caucasian descendant, with a previous history of hyperthyroidism, currently untreated, no symptoms of thyrotoxicosis with hypokalemic periodic paralysis.

CASE REPORT

Male patient, 33 years old, healthy, Caucasian, born and raised in Curitiba - Paraná. Prior to the first thyrotoxyc crisis, he didn't have any personal confirmed history of hyperthyroidism and no other comorbidities or use of alcohol and drugs. He was admitted by direct search to the Emergency Room of Hospital Universitário Evangélico Mackenzie due to proximal paralysis that started in the left side of the body and progressed to loss of global strength, in addition to two episodes of syncope.

He states that the acute symptoms started 1 week ago with the presence of asthenia and dizziness. After 2 days, he began to present dyspnea on minor exertion (walking approximately 50 meters). In the first hours of our hospital stay, the patient had paralysis of the proximal muscles of the left arm and leg, which progressed to paraplegia associated with global paresthesia. He also had two episodes of syncope witnessed by attending physician, lasting a few minutes, accompanied by bradycardia of 33 beats per minute (verified by finger oximeter), without release of sphincters or presence of muscle twitching.

He also mentioned have sought medical care twice in the past week with symptoms of anxiety, insomnia, tremors and weight loss of 16 kg that started in the last 4 months. Methimazole

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was prescribed due to clinical suspicion of thyroid alteration. During this investigation, a cranial computed tomography scan was performed, without any abnormality. The patient abandoned the treatment because he did not obtain the desired results.

On physical examination, the patient was lucid, oriented, with isochoric and photoreactive pupils, tachycardia (HR=118), normal blood pressure (120x76 mmHg) tachypnea (25 respiratory incursions per minute), with peripheral oxygen saturation of 95%, on room air. Absence of goiter or exophthalmos in the physical exam

The neurological examination showed preserved superficial and deep sensitivity, however, with altered motricity in the limbs. Strength in the lower limbs was classified as grade 0 in the proximal muscles and grade II in the distal muscles The upper limbs, on the other hand, presented grade III strength (absence of movement against resistance) in the distal muscles.

Laboratory tests requested at the emergency room showed serum TSH levels below 0.001 mU/L, free T4(FT4) of 4.42 ng/dL and initial potassium of 1.8 mEq/L. Due to the severity of the condition, intravenous potassium replacement was immediately started at a rate of 8.3 mEq/h (100 mEq of potassium in 12 hours).

The neuromuscular condition showed complete recovery from flaccid tetraplegia with normalization of serum potassium (5.4 mEq/l). The IV potassium replacement was switched to oral therapy, as well as medication to treat hyperthyroidism as propranolol 40 mg every twelve hours and metimazole 30 mg daily.

For diagnostic confirmation and etiological suspicion, a thyroid ultrasound (US) was requested, which revealed chronic thyroid disease with diffuse increase in vascularity on Doppler.

The patient was discharged from the institution after 48 hours of hospitalization, with a prescription of propranolol 80mg/day, metimazole 30mg/day and an outpatient request for antithyroid antibody dosages (anti-TPO, anti-TRAB), FT4, FT3 to confirm the diagnosis of Graves' disease

After 12 days of discharge, the patient returned to the emergency room complaining of oppressive chest pain in the retrosternal region and a feeling of heart palpitations. He also had pruritus and hyperemia on the right shoulder, hip and neck, compatible with urticaria without angioedema of probable pharmacodermic etiology. The admission electrocardiogram showed no abnormalities suggestive of acute coronary syndrome.

The conduct at the time of the evaluation was the suspension of metimazole (allergenic agent) and symptomatic prescription of promethazine 50mg IM and hydrocortisone 10mg IV with stabilization of the clinical condition. The patient was discharged with a home prescription of loratadine, if necessary, and referral to the cardiology and endocrinology team.

A transthoracic echocardiogram and exercise test were performed on an outpatient basis by the cardiology team to investigate chest pain, which did not show signs suggestive of ischemia. During consultation at the endocrinology outpatient clinic, 100mg propylthiouracil (PTU) was started every 8 hours, bilastine 20 mg/night for 10 days, and the use of prednisone 20mg for 5 days was advised.

At the outpatient return visit the patient already showed improvement in the urticaria, had propranolol reduced to 40 mg/day and had a plan to maintain the PTU for 12 to 18 months. TRab was positive confirming the diagnosis of Graves' disease.

Seven months (December) later the patient attended the outpatient clinic reporting a return of symtoms of anxiety and insomnia. He denied alterations in the evacuation pattern or other complaints and presented a weight gain of 3 kg since the previous attendance. So, new laboratory tests were requested (TRab, FT4, TT3, FT3 and TSH), and previous medications were maintained.

We must report that the patient was remiss about his treatment, not performing the hormones tests, not attending the medical appointments and probably not using the medications correctly.

Blood Count

	Hemoglobin (g/dL)	Leukocytes (1/mm³)	Platelets (1/mm³)
May 29	14,4	11.200	257.000
May30	13,3	9.270	190.700
May 31	15,2	6000	228.000
Jun. 14	15,8	19.500	268.000

Electrolytes

	Sodium (mEq/L)	Magnesium (mEq/L)	Phosphate (mEq/L)	Calcium (mEq/L)	Potassium (mEq/L)
May 29	135				1,8
May 30	141	1,99	3,92	5,2	5,4 (then 5,28 and 4,05 throughout the day)
Jun. 14	133			5,3	4,64

Thyroid function

	T4 (ng/dL)	TRAb (UI/L)	TPOab (UI/L)	TSH (µIU/mL)
Jun. 01	3,23			
Jun. 02		12,4 (reag.)	1,5	0,001
Jun. 09	0,48			2,01

Inflammatory function tests

	CRP (C-reactive protein) (mg/L)	ESR (Erythrocyte Sedimentation Rate) (mm/h)
May 29	0,11	
May 30		22
Jun. 09		2
Jun. 14	5,35	

Liver function tests

	AST (U/L)	ALT (U/L)
May 31	29,40	39,00
Jun. 14	23,10	32,00

Kidney function

	Urea (mg/dL)	Creatinine (mg/dL)
May 29	59,5	0,77
May 30	41,1	0,77
Jun. 14	32,60	0,93

Venous blood gases

	pH	pCO2 (mmHg)	pO2 (mmHg)	HCO3 (mEq/L)	BE
Jun. 14	7,38	47	20	27,8	2,7

Complementary Exams

Data	Exam	Report
May 29	Skull CAT	Thomographic study within normal limits.
May 31	Thyroid USG	Signs of chronic thyroid disease, with diffuse increase in vascularity on Doppler. Total volume 9.5cm³.
Oct. 17	Echocardiogram with Color Doppler	Echocardiogram within normal limits (Additional data see table below).
Dec. 12	Exercise Test	Effective response test (93%) not suggestive of myocardial ischemia.

Echocardiography with Color Doppler

	Measure	Reference for men
Left Atrium (AP Diameter)	34	
Left Atrium Volum Index	22	16 – 34 ml / m²
Aortic root	32	31 – 37 mm
RV Diastolic Diameter (RVOT)	25	20 – 30 mm
LV Diastolic Diameter	44	42 – 58 mm
LV Systolic Diameter	26	
Interventricular septum	07	06 – 10 mm

DISCUSSION

Thyrotoxic hypokalemic periodic paralysis (THPP) was initially described in the 19th century as a possible condition of hysteria and was only linked for the first time to hyperthyroidism in Germany, in 1902. It is an underdiagnosed disease due to its rarity and clinical similarity with other diseases that cause acute muscular paralysis (3,4). The largest number of cases occurs in Asian patients, with an incidence of 1.8 to 1.9% of patients with thyrotoxicosis, being uncommon in whites and blacks (0.1 to 0.2%). Although hyperthyroidism is more commonly diagnosed in women, THPP affects 20 times more men, mainly between 20 and 40 years. Our patient is, in fact, male and in the most prevalent age group, but he is not of Asian ethnicity (1,5). Clinical manifestations may occur in patients with a previous diagnosis of hyperthyroidism or as the first manifestation of thyroid alteration. The most frequent prodromal symptoms are tachycardia, pain and muscle stiffness. In the case in question, the patient had been presenting characteristic symptoms (weight loss, insomnia, agitation and tremors) for 4 months, and the diagnosis was made 4 days before admission. Although changes in sensitivity are not common, the patient complained of paresthesias associated with paralysis (6).

The main symptom of THPP is self-limited and recurrent muscle paralysis, which preferentially affects the proximal muscles of the lower limbs, as occurred with our patient. The laboratory tests show hypokalemia, sometimes hypophosphatemia and mild hypomagnesemia (3).

Paralysis may vary from minor weakness to flaccid paralysis, mostly proximal skeletal (3), which may last from hours to days, associated with concomitant hypokalemia (K < 2.5 mEq/L) (7). The patient in question had a potassium of 1.8 mEq/L. This, added to the complaints of generalized paralysis, is compatible with the definition of the disease.

The pathogenesis is still not well understood in the literature. However, it is believed that hypokalemia results from intracellular translocation of potassium (K+) due to hyperactivity of the Na+-K+ATPase pump, as a consequence of thyrotoxicosis. The stimulation of the pump is caused by β2-adrenergic agonists, amplifying the intracellular production of cAMP. The severity of hypokalemia correlates clinically with the degree of muscle weakness, which can even affect swallowing ability and respiratory activity (3).

The diagnostic suspicion occurs with the clinical manifestation of acute paralysis, related to precipitating factors, such as strenuous exercises and excessive consumption of carbohydrates and poorly controlled hyperthyroidism. Confirmatory diagnosis occurs when elevated thyroid hormones, thyroid-stimulating hormone suppression, and low serum potassium levels are found (1).

The diagnosis is based only on clinical and laboratory criteria, without an exam that presents specificity for the disease, it is

necessary to exclude other causes of recurrent muscle weakness, such as Guillain-Barré syndrome, familial hypokalemic periodic paralysis, myasthenia gravis, electrolyte disturbances and others neurological or muscular diseases (1).

As the disease can be fatal, as soon as the suspicion is confirmed, drug therapy and patient monitoring should be started. Emergency treatment consists of correcting hypokalemia through oral or intravenous potassium replacement and control of thyrotoxicosis using propranolol, non-selective beta-blocker that acts on adrenergic stimulation and the Na+-K+ATPase pump and in the peripheral deiodination of T4 in T3 (6,7). When serum potassium levels are normalized, muscle function is restored, as shown by our patient (2,3). The definitive treatment for preventing recurrences is based on the use of anti-thyroid drugs for 18-24 months. In case of relapses, opt for radioactive iodine or thyroidectomy (1).

CONCLUSION

This report aims to review the diagnosis, treatment and complications of hypokalemic periodic paralysis, as it is an underdiagnosed condition due to its rarity. In addition, the need for regular control of thyroid function is emphasized, in view of the potential cardiological and neurological involvement in cases of decompensation of this gland.

References

1. Valadão AF, Oliveira NF, Oliveira AA, Cajaíba LL, Neto, RPDC, Carvalho ND. Paralisia Periódica Hipocalêmica Tireotóxica: relato de caso. **Rev. méd. Minas Gerais**. 2021; p. 31411-31411.
2. Grzesiuk AK, Pinheiro MM, Neto NF, Rosa EN. Paralisia periódica hipocalêmica como primeiro sintoma de hipertireoidismo: relato de caso. **Arquivos de Neuro-Psiquiatria**. 2002; v. 60, n. 2B, pp. 490-492.
3. Dias, JCA, Moura BS, Gomes EF, Mirachi GB, Filho OM, Dias CBC. Paralisia periódica hipocalêmica tireotóxica: relato de 3 casos. **Arquivos Brasileiros de Endocrinologia & Metabologia**. 2004; v. 48, p. 897-902.
4. Borges NV, Bueno CV, Horta AL, Almeida DR, Shimonishida FS, Paixão PB, Vidal VG, Pires AC: Paralisia Periódica Hipocalêmica tireotóxica: relato de caso. **Rev. Soc. Bras. Clin. Med**. 2015;13(2):131-3.
5. Maia, ML, Trevisam PGC, Minicucci M, Mazeto GMFS, Azevedo, PS. Paralisia periódica hipocalêmica tireotóxica em paciente de ascendência africana. **Arquivos Brasileiros de Endocrinologia & Metabologia**. 2014; v. 58, p. 779-781.
6. Nazir M, Hameed M, Shehzad R Nazir, Mohsin. Thyrotoxic hypokalemic periodic paralysis: A success story of a diagnostic challenge. **Cureus**. 2021 ; v. 13, n. 4.
7. Statland JM, Fontaine B, Hanna MG, Johnson NE, Kissel JT, Sansone VA, Shieh PB, Tawil RN, Trivedi J, Cannon SC, Griggs RC . Review of the diagnosis and treatment of periodic paralysis. **Muscle & nerve**. 2018; v. 57, n. 4, p. 522-530.

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