مجلة الدراسات التربوية والعلمية - كلية التربية - الجامعة العراقية
العدد الثاني والعشرون - المجلد الأول - علوم الحياة - أيلول 2023 م
doi.org/10.52866/esj.2023.01.22.11

Hyperthyroidism and TNF-α Levels at Different Periods of Treatment

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Abstract :

Tumor necrosis factor- alpha (TNF-α) is a cytokine that has pleiotropic effects on immune cell types and non-immune cells. It has been identified as a major regulator of inflammatory responses, The level of TNF-α in hyperthyroid patients treated with carbimazole drug for different periods were determined. The total number of participants in the study was 115 male and female individuals with average age from 35 to 70 years old. They included: negative control group of thirty euthyroid persons represent group1, twenty-five hyperthyroid patient (control positive) included in the second group, thirty hyperthyroid patients treated with carbimazole drug for one year or less comprised the third group and thirty hyperthyroid patients treated with carbimazole drug for four year or more included the fourth group. By vein puncture, blood was collected and serum was isolated and preserved at –20 C. TNF-α were estimated using ELISA method. The results demonstrated that the level of TNF-α was highly significant ($p<0.01$) decrease in hyperthyoid patients as compared with negative control group. Also, there was highly significant ($p<0.01$) decrease in the level of TNF-α in treated groups (G3 and G4) with carbimazole drug in comparison to negative control group (G1). However, there was non-significant ($p<0.05$) difference in the level of TNF-α in hyperthyroid patient treated with carbimazole drug in group3 and group4 in comparison to hyperthyroid patients (G2).These findings suggest that the level of TNF-α decrease in hyperthyroid patient and still decreased after treatment with carbimazole drug for one year or less and for 4 years or more.

keywords: TNF-α, hyperthyroidism, carbimazole, drug.

فرط نشاط الدرقية ومستوى عامل نخر الورم في فترات زمنية مختلفة للمعالجة

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مستخلص:

عامل نخر الورم الفا هو سايتوكاين له تأثيرات متعددة الاتجاهات على أنواع الخلايا المناعية والخلايا غير المناعية. تم تحديده كمنظم رئيسي للاستجابات الالتهابية ، وتم تحديد مستوى عامل نخر الورم في المرضى المصابين بفرط نشاط الغدة الدرقية المعالجين بعقار الكاربيمازول لفترات زمنيه مختلفة. بلغ العدد الإجمالي للمشاركين في الدراسة 115 من الذكور والإناث بمتوسط أعمارهم من 35 إلى 70 عامًا. تضمنت: مجموعة السيطرة السالبة المكونة من ثلاثين شخصًا من الاشخاص اسوياء الغدة الدرقية يمثلون المجموعة 1 ، وخمسة وعشرون مريضًا بفرط نشاط الغدة الدرقية (السيطرة الموجبة) تمثل المجموعة الثانية ، وثلاثون مريضًا بفرط نشاط الغدة الدرقية المعالجين بعقار الكاربيمازول لمدة عام واحد أو أقل (المجموعة الثالثة) وثلاثون مريضًا بفرط نشاط الغدة الدرقية المعالجين بعقار الكاربيمازول لمدة اربعة اعوام أو أكثر يمثلون المجموعة الرابعة .عن طريق ثقب الوريد ، تم جمع الدم وعزل المصل وحفظه عند درجة حرارة – 20 درجة مئوية. أظهرت النتائج انخفاضا معنويًا ($p <0.01$) بدرجة عالية في مستوى عامل نخر الورم الفا في مرضى فرط نشاط الغدة الدرقية. كذلك، كان هناك انخفاضا معنويًا ($p <0.01$) بدرجة عالية في مستوى عامل نخر الورم في المجموعات المعالجة (الثالثة والرابعة) بعقار الكاربيمازول مقارنة مع السيطرة السالبة (المجموعة الاولى). ومع ذلك ، كان هناك اختلاف غير معنوي ($p <0.05$) في مستوى عامل نخر الورم الفا في مرضى فرط نشاط الغدة الدرقية الذين عولجوا بعقار الكاربيمازول في المجموعة الثالثة والمجموعة الرابعة مقارنة بمرضى فرط نشاط الغدة الدرقية في المجموعة الثانية، وتشير النتائج إلى أن مستوى عامل نخر الورم الفا ينخفض في حالة مرضى فرط نشاط الغدة الدرقية واستمرار انخفاضها بعد العلاج بعقار كاربيمازول لمدة عام أو أقل ولمدة 4 سنوات أو أكثر.

الكلمات الرئيسية: عامل نخر الورم الفا، فرط نشاط الغدة الدرقية ، كاربيمازول ، دواء.

Introduction:

Thyroid gland is one of the largest endocrine glands in the body which plays a major role in metabolism, growth and development of human body. It also helps to regulate many body functions by constantly releasing a steady amount of thyroid hormones into the bloodstream. Imbalance of thyroid hormones can lead to either hypo or hyperthyroidism [1]. Hyperthyroidism is an excessive concentration of thyroid hormones in tissues caused by increased synthesis of thyroid hormones, excessive release of preformed thyroid hormones, or an endogenous or exogenous[2]. In addition to toxic multinodular goiter and toxic adenoma, Graves' disease; the most common cause of hyperthyroidism, is an autoimmune disorder in which thyroid-stimulating antibodies activate thyroid-stimulating hormone (TSH) receptors, triggering thyroid hormone synthesis [3].

Antithyroid drugs (ATDs), radioactive iodine ablation, and surgery are the three options for treating patients with hyperthyroidism. These therapeutic options would be effective in the treatment of patients with GD, whereas patients with toxic multinodular goiter or toxic adenoma should have either radioactive iodine therapy or surgery [4].

Over the past eight decades, the thionamide drugs, i.e. carbimazole and its metabolite methimazole (MMI), and propylthiouracil (PTU) have extensively been used in the management of various forms of hyperthyroidism. Carbimazole is obtainable in some Asian and European countries and is converted to the active form, methimazole, with similar properties to methimazole [5].

Carbimazole is a pro antithyroid drug that belongs to thioamide group (Carbimazole, Methimazole and propylthiouracil). After converted to its active form, MMI prevents thyroid peroxidase enzyme from iodinating and coupling the tyrosine residues on thyroglobulin, hence reducing the production of the thyroid hormones T3 and T4 [6].

In patients with thyroid dysfunction, changes in lipids profile and adipokines have been recorded. Thyroid dysfunction affects the lipids profile and adipokines [7].

Cytokines are small proteins with a low molecular weight that control the interaction between tissues and the im-

mune system. Many different cell types produce these molecules, which play important roles in health and disease. Cytokines may be used as diagnostic, prognostic, and therapeutic biomarkers for health and disease [8].

Tumor necrosis factor alpha (TNF-*α*) is a cytokine that is released by inflammatory cells and adipocytes in response to chronic inflammation [9]. This inflammatory cytokine produced by macrophages/monocytes, endothelial cells, smooth muscle cells, neutrophils activated lymphocytes, and adipocytes during acute inflammation and is responsible for a various range of signaling events within cells, leading to necrosis or apoptosis. One of important functions for TNF-α is mediating expression of genes for receptors, growth factors, transcription factors, cytokines. Moreover ,the protein is important for resistance to infection and cancers [10].

The production of IFN-γ and TNF-α in GD is mediated by recruited Th1 lymphocytes which enhance Th1 chemokines release from thyrocytes, initiating and continuing the autoimmune process. The active phase of GD is associated with these circulating chemokines. If there is high level of Th1 chemokines in peripheral fluids, this will be a marker for the host immune response [11].

By adipocytes and other cells in the tissue matrix, TNF-α is produced in adipose tissue. Adipocyte production and blood levels of TNF-α related with hyperinsulinemia and body max index (BMI) , besides TNF-α impairs insulin action by inhibiting insulin signaling [12].

In this study, The levels of proinflammatory cytokine TNF-α was investigated in the serum of hyperthyroid treated patient with carbimazole drug at different periods to inspect the possible effects of this drug during treatment periods.

Materials and Methods

Sample collection and study group deign

The total number of participants in the study was 115 male and female individuals with an average age from 35 to 70 years. All samples were obtained from both Al–Mustansiryah University; National Diabetes Center and Specialist Center for Deaf diseases and Diabetes glands from December 2019 to

December 2020. They were diagnosed by hyperthyroidism according to symptoms and hyperthyroidism control test score.

- :The study groups were as follows

Group 1: Thirty Euthyroid healthy individuals of both sexes at different ages with no treatment and considered as negative control.

Group 2: Twenty-five male and female hyperthyroidism patients with no treatment, so it was considered as pos-.itive control

Group 3: Thirty patients diagnosed with hyperthyroidism treated with carbimazole drug for one year or less.

Group 4: Thirty male and female patients with hyperthyroidism treated with carbimazole for 4 years or more.

Patients with diabetic mellitus, hypertension, autoimmune disease, kidney and liver disease were excluded from the study

About 5 ml of peripheral whole blood was aspirated from each controls and patient groups using plastic disposable syringe. The blood was transferred into gel tube, and in water bath, allowed to clot for ten minutes at 37C °, at that moment were centrifuged at (3000 rpm) for 10 minutes. Then clear serum was obtained and stored frozen at -20 C°until being used for adipokines parameters assay. The hemolyzed samples were discarded [13].

Detection of the levels of TNF-α

In human serum, TNF-α level were detected depending the manufracturer instructions of kits (Bioassay Technology Laboratory, china), (Bioassay Technology Laboratory, china) respectively using ELISA method. The standard curve for vaspin and obestatin were illustrated in Fig1 and Fig2.

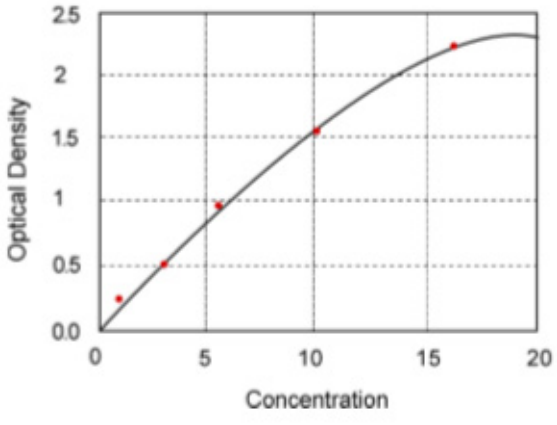

Fig 1: standard curve for Vaspin

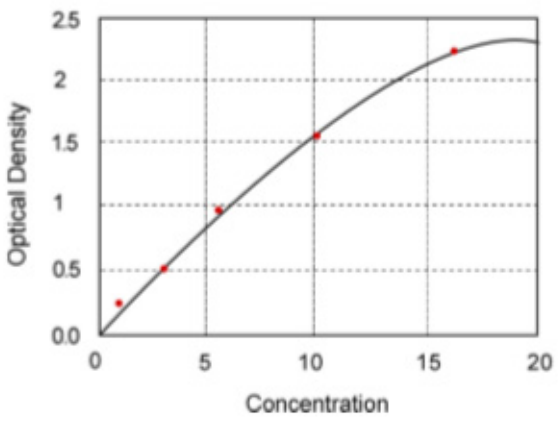

Fig2: Standard curve for Obestatin

Statistical analysis:

To detect the impact of different factors in study parameters, the Statistical Analysis System- SAS (2012) software was used. To make a significant comparison between means, the T-test and the Least Significant Difference –LSD test (ANOVA) were used [14].

Results and Discussions

Levels of TNF-α

In table (1) the mean concentration of serum TNF-α were measured in all studied groups and compared between the healthy group and patients.

The concentration of TNF-α was highly significant ($p<0.01$) decrease in G4 (43.73 ±3.19 ng\L) in comparison with G1(74.48 ±4.47 ng\L). The results exposed there was no significant ($p>0.01$) difference in G4 as compared with positive control and G3 (50.19 ±3.25ng/L ,49.01 ±11.44ng/L, respectively). Also there was a highly significant ($p\leq 0\ 01$) decrease in TNF-α in G3 (49.01 ±11.44ng/L) in comparison with negative control (74.48 ±4.47 ng/L).While there was no significant ($p>0.01$) difference in G3 as compared with positive control.

Table 1: Compare between different groups in TNF-α levels.

TNF-α levels for all groups	Mean ± SE
Group1	74.48 ±4.47 **a**
Group2	50.19 ±3.25 **b**
Group3	49.01 ±11.44 **b**
Group4	43.73 ±3.19 **b**
LSD value	18.358 **
p-value	0.0059
Means having with the different letters in same column differed significantly.** ($P\leq 0\ 01$) .	

Tumor necrosis factor alpha and TNF- receptors have been found in thyroid follicular cells, and they are thought to be involved in the cytotoxic mechanisms that cause thyroid damage in autoimmune thyroid disease. TNF-α levels in the blood have been shown to be elevated in gravies disease (GD) patients, and TNF-α administration to humans has been shown to cause hor-

monal changes. These findings support the importance of TNF-system activation in patients with thyroid dysfunction. The treatment of hyperthyroidism is followed by a substantial decrease in previously elevated TNF-α and its receptors concentrations[15]. The findings of [16] also support the idea that polymorphism in pro-inflammatory cytokines may play a role in GD predisposition. It's worth noting that some antithyroid medications have antioxidant properties. Methimazole and propylthiouracil both inhibited or reduced the production of radicals by complement-attacked thyroid cells, as well as cytokine production [17]. Inhibition of TNF– expression in carbimazole-treated patients could be a direct anti-inflammatory effect. This may be due to carbimazole's inhibitory effect on Rac1, which is involved in TNF-expression [18]. In GD, recruited Th1 lymphocytes are responsible for enhanced IFN-γ and TNF-α production, which in turn stimulates Th1 chemokine's release from thyrocytes, initiating and perpetuating the autoimmune process circulating levels of these chemokine's are associated with the active phase of GD. Environmental factors play a role in the diagnosis of the disease to the tune of 30%. Stress, which has long been thought to be a potential trigger for GD, is one of the environmental factors at the root of the disease [19]. Only a few studies examining the role of serum cytokines in hyperthyroidism have considered the role of age, which appears to be a determinant variable that is substantially and directly correlated with several cytokines such as TNF-α [20]. The active phase of GD (newly diagnosed and relapsing) was linked to serum chemokine levels, and their reduction in GD patients treated with MMI could be linked to the immunomodulatory effects of MMI [21]. The effects of carbimazole on the expression of inflammatory markers caused by a high-fat diet, as well as the relationship between obesity, thyroid function, and inflammation was studied by [22]. They demonstrated that induction of TNF- was significantly inhibited by carbimazole treatment, without affecting other parameters. In primary human T-lymphocytes, it has found that the heterocyclic thionamides carbimazole and propylthiouracil inhibit the development of the proinflammatory

cytokines tumor necrosis factor (TNF) alpha and interferon (IFN)gamma. Furthermore, nuclear factor (NF)-kappaB DNA binding, a proinflammatory transcription factor that regulates both TNFalpha and IFNgamma synthesis, as well as NF-kappaB-dependent reports [23].

Acknowledgments

We are thankful to all participants in this study and also to the staff of National Diabetes Center; Al–Mustansiryah University and Specialist Center for Deaf diseases and Diabetes glands .we did not get the financial source of the study.

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