Multiple Sclerosis is a Risk Factor for Hyperthyroidism and Interferon Beta Action on Thyroid Hormones via Novel Immuno-neuro-enzymological Mechanisms

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Abstract:
Objective: Multiple sclerosis (MS) is a common neurological disease deeply linked with the immune-inflammatory disorders whereas the term (multiple) mostly refers to the multi-focal zones of inflammation caused by lymphocytes and macrophages infiltration besides oligodendrocytes death. Accordingly, the dysfunctional immune system able to damage myelin (a pivotal component of the central nervous system) which responsible for communication among neurons. The aim of the present study is to innovate a biochemical relationship between MS and thyroid hormones (THs) by highlighting immunological responses and also to examine the action of Interferon beta (IFNβ) drug on thyroid hormone (THs) and thyroid stimulation hormone (TSH). Materials and methods: Sixty (60) Iraqi women in the age ranged (36-43) years were enrolled in the present study, (30) of them were MS patients and the other (30) were healthy. Anyway, the protocol of the study involved four groups: G1 is a healthy control group, G2 involved untreated MS patients, G3 included the MS patients treated with IFNβ for (6) weeks and G4 composed of the same patients treated with IFNβ for (12) weeks. THs (T4 and T3) and TSH levels were determined in sera of all groups. Results: Data of the present study have reported that T4 level was highly significant increase in sera of G2 compared with G1 while it was significant and highly significant decreased in G3 and G4 respectively compared with G2, the difference between G4 and G1 and also between G4 and G3 was significant. T3 level was highly significant increase in sera of G2 compared with G1 but it was highly significant decreased in G3 and G4 compared with G2, the difference between G4 and G1 was non-significant while the difference between G4 and G3 was significant. Conversely, TSH level was highly significant decreased in G2 compared with G1 but it was highly significant increase in G3 and G4 compared with G2, the difference between G4 and G1 and also between G4 and G3 was highly significant. Conclusions: Interestingly, the present study is the first in Iraq reporting that MS may be a key risk factor for hyperthyroidism and also the first suggesting that IFNβ regulates THs biosynthesis via novel immuno-neuro-enzymological mechanisms regarding thyroid peroxidase (TPO) and iodothyronine deiodinase 1 (D1), meanwhile the present study indicates that IFNβ has an indirect antioxidant activity. Moreover, the present study provides a definite clarification for the changed NF kappa B level in MS. Remarkably, the present study reveals that IFNβ is more potent on T3 than T4 while it has less action on TSH.

Key words: Multiple sclerosis. T4. T3
Introduction

Multiple sclerosis (MS) is a complex chronic auto-immune inflammatory disease [1,2,3] characterized by repeated demyelination of the central nervous system [4,5,6]. Demyelination is a pathophysiological condition including localized destruction of myelin [7,8]. Moreover, its pathophysiology involves oligodendrocytes death and axonal degeneration.[8] Lesions or plaques composed of inflammatory and demyelinating cells cross with the correct transmission of nerve impulses and cause neuronal dysfunction mainly autonomic and sensorimotor defects, difficulties in thinking and fatigue. [9] Broadly, MS is diagnosed by magnetic resonance imaging (MRI). [10,11] Thyroxine (T4) and triiodothyronine (T3) are the mostly hormones linked with metabolism, they are secreted by the thyroid gland, [12] under stimulation of thyroid stimulating hormone (TSH) which secreted by pituitary gland. [13] Moreover, thyroid hormones (THs) T4 and T3 controls a different essential biophysiology processes involving brain development, body growth, skeleton maturation, oxygen consumption, reproduction, heat production and heart contractility. [14] Thyroid stimulating hormone (TSH) is a major sensitive biochemical hormone reflecting thyroid function [15] because its secretion is being

الخلاصة:

الثدييات المتعددة هي إحدى الأمراض العصبية الشائعة المرتبطة بقوة مع الإضطرابات المناعية الإلتهابية حيث أن كلمة (متدف) غالبًا تعود إلى مصطلح موضعية متعددة من الإلتهاب بسبب تسرير وتأثير الخلايا المضادة والخلايا البلعمية. تشير النتائج إلى أن هذه الفئات الاقليائية الصغرى المضادة المعززة للخلايا البلعمية متعلقة مع الظروف الطبيعية والنظام المناعي للهربون عند الحيوانات. إن هذه الدواوي الاحتياطية من المضادات الحيوية المتعددة مثل أدوية الثيرونين والثايرونين مع مرحلة العصبين الفيسيولوجي يمكن أن تؤدي على المرضى.يبدو أن هذه الدواوي لا تؤثر على الوظائف العصبية والدماغية. تتطلب هذه الدواوي تحريصًا من خلال بالإستجابة المناسبة لتحديد وتطبيق الأدوية المناسبة. إذاً، يمكن أن يؤدي أتم الطرق الإستجابة إلى يعتبر في الحالة العصبية الإلتهابية.
under negative feedback control depending on THs. [16] Interferons (IFNs) are cytokines with protein structure, play a central role in the immunological responses. There are three types of IFNs used as drugs: IFNα, IFNβ and IFNγ. IFN is commonly used for MS treatment. [17] Accordingly, exogenous IFN (used as drugs) interacts with endogenous (natural) IFN by immune-modulations pathways. [18]

Materials and methods

• Basics of research:
Honestly, at the beginning of the present study, the first intention was to measure some biochemical parameters in MS patients rather than THs and TSH, but during blood samples collections in both of MS clinic of medical city / Baghdad and private laboratories I noted some features related to thyroid gland disorder in MS patients. Accordingly, this interested circumstance provoke a motive for me to change the direction of the research towards studying the relationship between untreated MS patients and thyroid gland disorders by determination THs and TSH, and in the same time examination the action of the drug given commonly to MS patients in Iraq (IFNβ).

• Subjects:
Sixty (60) Iraqi female with age in range (36-43) years were enrolled in this study, (30) of them were untreated MS diagnosed by magnetic radiation image (MRI), the other (30) were healthy (enrolled as a control group). Patients were treated with IFNβ injection (250 µgm/mL) one injection / two days for (12) weeks. The protocol of the present study involved four groups: G1: healthy control group (30 member). G2: untreated MS patients (30 patient). G3: MS patients treated with IFNβ for (6) weeks (30 patient). G4: MS patients treated with IFNβ for (12) weeks (30 patient). Practically, T4, T3 and TSH were determined in sera of each group.

• Blood samples and biochemical parameters determination:
Five milliliters (5mL) of venous blood were collected from all subjects in this study, placed into plain tubes, when coagulation was happened, serum was isolated by centrifugation (4000 round per minute) subsequently, sera were kept frozen until biochemical determination. THs (T4 and T3) were determined by competitive ELISA method, T4 contained in serum (the native antigen) competes with T4 horseradish peroxidase conjugate for binding with monoclonal antibody coated on the microplate. Also a competition reaction occurred between the native antigen of T3 and horseradish peroxidase conjugate to bind with a limited number of biotinylated antibody (Anti-T3 labeled with biotin) In both of T4 and T3 determination, the enzyme activity in the antibody bound fraction is measured by a reaction with a substrate and producing a color its intensity is inversely proportional with T4 and T3 concentrations. On the other hand, TSH is determined by sandwich ELISA method, high affinity and specific antibodies (enzyme and immobilized) were used and the native antigen is included within serum, a reaction happened between TSH antibodies and the native TSH in the microwell without a competition forming a soluble sandwich method, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium was attained, the antibody – bound fraction was separated from unbound antigen by decantation, enzyme activity in the antibody bound fraction is directly proportional to the native antigen concentration. Similarly, the enzyme activity presenting on the surface of the well was determined by a reaction with a substrate and producing a color its intensity proportional with TSH concentration.

• Statistical Analysis:
Data of the present study were represented as mean ± S.D (S.D : standard
deviation), student t-test was applied for estimation the degree of significance among groups by calculating probability value (p), (p< 0.001), (p<0.05) and (p≥0.05) were assumed statistically highly significant, significant and non-significant respectively as will be reported in results divisions.

**Results**

**Table (1): T₄ level in sera of MS patients and control subjects.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>T₄ level (nmol/L)</th>
<th>Mean</th>
<th>S. D</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>10.63</td>
<td>85.2</td>
<td>10.63</td>
</tr>
<tr>
<td>G₂</td>
<td>16.78</td>
<td>194</td>
<td>17.68</td>
</tr>
<tr>
<td>G₃</td>
<td>9.23</td>
<td>110</td>
<td>9.23</td>
</tr>
<tr>
<td>G₄</td>
<td>99.8</td>
<td>99.8</td>
<td>8.12</td>
</tr>
</tbody>
</table>

P G₂/G₁: H.S (1.16969E-17) P G₂/G₃: S (0.00154) P G₂/G₄: H.S (3.08629E-18) P G₁/G₃: S (0.0036) P G₁/G₄: S (0.00163)

Results of table (1) have reported that T₄ level was highly significant increase in sera of G₂ (194±16.78) nmol/L compared with G₁ (85.2±10.63) nmol/L, conversely, it was significant and highly significant decreased in sera of G₃ (110±9.23) nmol/L and G₄ (99.8±8.12) nmol/L respectively compared with G₂ (194±16.78) nmol/L while the difference between G₄ (99.8±8.12) nmol/L and G₁ (85.2±10.63) nmol/L was significant and the difference between G₃ (110±9.23) nmol/L and G₂ (194±16.78) nmol/L was significant. Also, results of table (2) have suggested that T₃ level was highly significant increase in sera of G₂ (3.42±0.75) nmol/L compared with G₁ (1.82±0.39) nmol/L but it was highly significant decreased in sera of G₃ (2.33±0.58) nmol/L and G₄ (1.90±0.47) nmol/L compared with G₂ (3.42±0.75) nmol/L, remarkably the difference between G₄ (1.90±0.47) nmol/L and G₁ (1.82±0.39) nmol/L was non-significant while the difference between G₄ (1.90±0.47) nmol/L and G₃ (2.33±0.58) nmol/L was significant. Nevertheless, results of table (3) have shown that TSH level was highly significant decreased in sera of G₂ (0.13±0.01) µU/mL compared with G₁ (2.10±0.12) µU/mL, interestingly it was highly significant increase in sera of G₃ (0.5±0.04) µU/mL and G₄ (1.04±0.07) µU/mL compared with G₂ (0.13±0.01) µU/mL but the difference between G₄ (1.04±0.07) µU/mL and G₁ (2.10±0.12) µU/mL was highly significant. Moreover, the difference between G₄ (1.04±0.07) µU/mL and G₃ (0.5±0.04) µU/mL was highly significant.

**Table (2): T₃ level in sera of MS patients and control subjects.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>T₃ level (nmol/L)</th>
<th>Mean</th>
<th>S. D</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>1.82</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>G₂</td>
<td>3.42</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>G₃</td>
<td>2.33</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>G₄</td>
<td>1.90</td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>


**Table (3): TSH level in sera of MS patients and control subjects.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>TSH level (µU/mL)</th>
<th>Mean</th>
<th>S. D</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>2.10</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>G₂</td>
<td>0.13</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>G₃</td>
<td>0.5</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>G₄</td>
<td>1.04</td>
<td>0.07</td>
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Discussion

Thyroid peroxidase (TPO) is the key enzyme responsible for the biosynthesis of T₄ and T₃. [19] which are secreted by the thyroid gland. [20] In particular, this enzyme catalyzes covalent incorporation of oxidized iodine into tyrosine in thyroglobulin and coupling of iodinated tyrosine to form T₄ and T₃. [21] thyroglobulin regulates development and function of the central nervous system. [22] According to multiple sclerosis which is characterized by inflammatory auto-immunological demyelinating conditions, [23] a recent study has revealed that tissue damage in MS resulted from a complex and dynamic interplay among immune cells, (myelin-making oligodendrocytes) and neurons. [10] In this regard, a recent study has suggested that oligodendrocytes are the major cause of demyelination in MS patients. [24] Also a second recent one has reported that B cells and T cells mostly contribute to the physiology of demyelinating disorders. [1] Moreover, a previous study has shown that demyelination may be mediated by a direct attack of macrophages. [25] Interestingly, a recent review has mentioned that myelin debris activates nuclear factor kappa B (NF-kB) in neural and glial cells mediating neuro-inflammation and neuro-degeneration, [26] NF-kB constitutes a major component in many immunological responses. [27] Consequently, the present study provides a definite pathway for the high expression and activity of NF-kB in MS patients by highlighting immunological factors that lead to demyelination. Although the recent study [2] suggested that NF-kB pathway is changed in MS, but the mechanism was partially understood. Since NF-kB is expressed primarily in the brain , [28] it plays a crucial role in the nervous system , [29] In accordance with thyroid hormones , NF-kB acts as a regulatory element in TPO expression , it has been suggested by [21] TPO promoter has a conserved binding site for NF-kB. Consequently, the over-expression of NF-kB resulting from demyelination in MS up-regulates TPO and subsequently induces T₄ and T₃ biosynthesis over the normal range (The major features of hyperthyroidism). On the other hand, reactive oxygen species (ROS) are so active in MS. [1] because those neuro-toxic molecules (produced by inflammatory cells) are present at an active demyelination site. [24, 30] Increased levels of ROS and their intermediates induce cellular damage and trigger chronic inflammation. [25, 31] Interestingly, Hydrogen peroxide H₂O₂ binds with thyroglobulin tyrosine to form iodothyronine in the first step of THs biosynthesis. [32 ,33] As a result, the present study submits a neuro-immuno mechanism emphasizing the data related to the highly significant increase of T₄ table (1) and T₃ table (2) in G₂ (untreated patients) compared with G₁ (healthy control subjects). This mechanism divided into two pathways: The first by up-regulation of TPO via over expression of NF-kB and the second by activation of TPO via higher level of H₂O₂, both of the two pathways linked with demyelination. In other words, the present study is the first reporting that MS may be a key risk factor for hyperthyroidism via novel immuno-neuro enzymological pathways, fig (1) Remarkably, T₄ and T₃ levels were depressed under treatment with IFNβ as shown in table (1) and table (2). However, the significant and highly significant decrease of T₄ in G₃ and G₄ compared with
G2 besides the highly significant decrease of T3 in G3 and G4 compared with G2 revealed that their biosynthesis was down regulated by IFNβ. Hence, immunological factors lead to demyelination (macrophages, lymphocytes) and oligodendrocytes in fig (1) must be highlighted. Broadly, endogenous IFNβ, is released by macrophages and activates the immunological responses through type I IFN signaling pathway, [34] exogenous IFNβ acts as indirect inhibitor for endogenous IFNβ signaling by overcoming the inhibition and rescuing infected macrophages. [35] Accordingly, a recent study has revealed that macrophages play a potential role in demyelination and remyelination. Hence, depletion of macrophages plays a pivotal role in decreasing the severity of demyelination. [24] Furthermore, IFNβ interacts with the immune system at multiple levels by modulationg the function of T cells and B cells in the adaptive immune system. Also, IFNβ help maintaining the number of type I interferon secreting plasmacyloid dendrocytes. [3] Consequently, when macrophages, lymphocytes and dendrocytes changed to the balance by the superior drug IFNβ, demyelination will be inhibited, subsequently NF-kB expression will be controlled, and TPO shifted towards its normal activity, in other words T4 and T3 biosynthesis will be down-regulated. Therefore, this study is the first reporting that IFNβ is an indirect regulator for THs in Iraqi patients with MS via its reactive effect on demyelination, fig (2). Moreover, IFNβ has an indirect antioxidant property caused by inhibition of demyelination and consequently restrict the sites of H2O2. However, the significant decrease of T4 table (1) and T3 table (2) in G4 compared with G3 suggesting that the duration of treatment with IFNβ has a positive action. The difference between G4 and G1 in T3 table (2) was non-significant while it was significant in T4 table (1) revealing that T3 approximately reached its normal levels under treatment with IFNβ. In accordance with T3, iodothyronine deiodinase plays a central role in maintaining normal serum T3. [36] Remarkably, a relationship between IFNβ and iodothyronine deiodinase may be possible. Anyway, 20% of T3 is directly released by the thyroid gland under normal bio-physiological circumstances, [21] and predominantly 80% of T3 derived from T4 metabolism by iodothyronine deiodinase. [14] Besides TPO, T3 levels and its biosynthesis is controlled by iodothyronine deiodinase, [37] there are three types of iodothyronine deiodinase: iodothyronine deiodinase 1 (D1), iodothyronine deiodinase 2 (D2) and iodothyronine deiodinase 3 (D3), they catalyze the production and degradation of T3 respectively via the outer and inner deiodination. [38] Interestingly, D1 is expressed not only in thyroid and other organs linked with metabolism, [39] but also in lymphocytes (B cells and T cells) as mentioned by the recent study [37], that also has suggested a correlation between D1 and inflammation. Moreover, the study [37] linked between neurological disorders (brain and depressive disorders) and high expression of D1. Consequently, D1 is highly expressed in MS patients where the immune system is destroyed and neurological disorders are possible, fig (2). On the other hand, IFNβ modulates inflammatory – immunological responses related to B cells and T cells and depress pro-inflammatory cytokines (although exogenous IFNβ is also a cytokine but it acts as anti-inflammatory cytokine). [40,
As a result, D_{1} is down-regulated by IFNβ towards the balance and T_{3} will be approximately reached the normal balance in G_{4} table (3). Collectively, IFNβ could be considered as indirect down-regulator for D_{1} in MS, fig (3). Lastly, according to table (3), the highly significant decrease of TSH in G_{2} compared with G_{1} indicate that untreated MS suffering from hyperthyroidism (besides higher level of T_{4} and T_{3}) cited above, this significant decrease of TSH in G_{2} compared with G_{1} and the highly significant increase in G_{3} and G_{4} compared with G_{2} are explained based on T_{4} and T_{3} level in the context of pituitary thyroid feedback regulation. [43, 44] When T_{4} and T_{3} biosynthesis is overactive, TSH releasing will be suppressed by the negative feedback inhibition. In turn, when T_{4} and T_{3} levels in blood decreased, the pituitary release more TSH. [45, 46] Another recent study emphasizes the last explanation related to TSH by reporting that lower level of TSH mostly refers to the over-activity of thyroid gland (hyperthyroidism). [47] The highly significant difference between G_{4} and G_{1} suggested that IFNβ has a less action on TSH in comparison with T_{4} and T_{3} whereas the highly significant difference between G_{4} and G_{3} emphasizes the role of treatment duration.

Figure (1) : The suggested mechanism for MS as a risk factor for hyperthyroidism via the up-regulation of TPO

Figure (2): The indirect action of IFNβ as a down-regulator for TPO.
Conclusions
This study is the first in Iraq reporting: 1) Untreated MS may be a key risk factor for hyperthyroidism via novel immuno– neuro– enzymological mechanism regarding TPO dividing into two pathways: the first including the over-expression of NF-kB as an up-regulator for TPO and the second dealing with higher levels of H2O2 as a cofactor for TPO. 2) A definite clarification for the changed level of NF-kB in untreated Iraqi MS patients based on immuno-neuro changes. 3) A novel immuno–neuro- enzymological mechanism by which IFNβ modulates THs biosynthesis in Iraqi MS patients via the indirect down regulation of TPO. 4) An immuno – neuro relationship between untreated Iraqi MS and high expression of D1. 5) An indirect mechanism by which IFNβ down-regulate D1 activity and shifting T3 towards the balance in Iraqi patients with MS.

References


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