ASSOCIATION OF SERUM AUTO-ANTIBODIES AND THYROID FUNCTION TEST IN PATIENTS WITH NON-NEOPLASTIC THYROID DISEASES IN SHENDI LOCALITY, SUDAN

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ABSTRACT

Background: Thyroid disorders are the most second endocrine problem after diabetes mellitus in society. Environmental, immunological, and genetic factors lead to the development of thyroid disorders.

Objectives: The study intended to evaluate the role of thyroid antibodies and hormones in the diagnosis of thyroid diseases, as well as the prevalence of thyroid antibodies in patients having thyroid disorders living in Shendi locality.

Study design: The current research was prospective, case-control, hospital-based study carried out from 2013-2017, in El-Mek Nimir University Hospital in Shendi town - Northern Sudan. River Nile State. Two hundred and eighty-three (283) participants selected randomly as study population, of whom Hundred and eleven (111) patients with hypothyroidism; Seventy-two (72) patients with hyperthyroidism; Hundred (100) healthy normal subjects as control group. Participants were recruited from outpatient clinics in El-Mek Nimir University Hospital in Shendi Thyroid hormones profile (TSH, T4, T3, fT3, and fT4) and Thyroid antibodies (Anti-thyroid peroxidase and antithyroglobulin) were measured.

Results: The study revealed that (60.7%) had hypothyroidism, (39.3%) had hyperthyroidism, (91.9%) of hypothyroidism were female, and only (8.1%) were male, while (84.7%) of hyperthyroidism were female while male represented only (15.3%) of them. The current study revealed that 107 of the case group (58.5%) were positive when evaluated for thyroid peroxidase antibodies (TPO Ab), with level more than (40.0 IU/ml). 72 (64.9%) of them were hypothyroidism, whereas a 35 (48.6%) of them were hyperthyroidism. As regard Thyroglobulin antibodies (Tg Ab), 73 (39.9%) of the case studied were positive, 51 (69.9%) of them were hypothyroidism, and 22 (30.1%) of them were hyperthyroidism. The study also revealed statistically significant positive correlation between the presence of TPO Ab and the values of fT3.

Keywords: Thyroid Gland, Thyroid Hormones, Thyroid Dysfunction, Hypothyroidism, Hyperthyroidism, Thyroid Autoantibodies, Tsh, Tt4, Ft4, Tt3, Ft3, Tpo Ab, Tg Ab

1. INTRODUCTION

Thyroid disorders constitute the main bulk of endocrine diseases second to Diabetes Mileus Jayakumar (2011). Thyroid disease usually occurs between (30 to 50) years of age. Overt hyperthyroidism occurs in about (20 per 1000) women and (2 per 1000 men). Health Informatics at Newcastle. (1999). The natural trace element Iodine is an essential micronutrient needed for normal thyroid function,
linear growth, and brain development. Less than required iodine intake compromised thyroid function WHO (2010). Disorders of iodine deficiency are a significant global problem even in the well-developed counties such as the United States of America (U.S.) Wu et al. (2002). For the first time, thyroid hormone was isolated in the year 1914 by Kendall Kendall (1919), and latter firstly synthesized in the year 1925 by Harrington Harrington (1926). T4 is the principal hormone secreted by the thyroid gland. It is about (~80%) of the thyroid hormones secreted in human, the remaining (~20%) is triiodothyronine T3. T3, is regarded as the primary biologically active form, since T4 has limited affinity for the nuclear thyroid hormone receptors. T4 has to be converted to T3 by outer ring deiodination. Both T4 and T3 can be inactivated by inner ring deiodination. These reactions are catalyzed by the iodothyronine deiodinases type 1, 2 and 3 (D1, D2 and D3), expressed in a multitude of peripheral tissues, Bianco et al. (2002). Evaluation of thyroid function is carried out by measurement of thyroid stimulation hormone (TSH) as the main test Dufour (2007). The majority of cases of primary overt thyroid diseases can be excluded if TSH value is within the reference interval limit. fT4 is measured for confirmation of diagnosis if TSH is abnormal Thyroid Foundation of Canada (2009). The serum level of TSH may be slightly elevated in the presence of normal level of fT4. Ogedebe (2007), therefore measurement of fT4 and fT3 are indicated other than that of TT4 and TT3 levels. Furthermore, checking of fT3 is not indicated in hypothyroidism. Int Assoc of Medical Laboratories (2007).

Thyroid peroxidase (TPO) is the key enzyme for the synthesis of the thyroid hormone, as it catalysed both the iodination and coupling reaction. TPO is membrane bound and found in the cytoplasm and in high concentration on the apical microvillar surface of thyrocytes. It has molecular weight of (100 to 150 KDa). It is known before as thyroid microsomal antigen, McLachlan and Rapoport (1992). Multiple T – and B – ce II epitopes exist within the molecule, and the antibody response to TPO is restricted at the level of the germ line heavy and light chain variable V region. McIntosh et al. (1998). In over (90%) of patients with autoimmune hypothyroidism and Graves’ disease, Anti-TPO autoantibodies are detected. Anti-TPO autoantibodies and thyroglobulin (Tag) antibodies are the predominant antibodies in autoimmune hypothyroidism. Anti-TPO antibodies are predominantly of the IgG class 1 and IgG4 subclasses in excess. Njemini et al. (2002), Hawa et al. (2006). Thyroglobulin (Tag) is glycoprotein of molecular weight (660-KDa) and composed of two identical subunits of (330 KDa) each. It is secreted by the thyroid follicular cells into the follicular lumen and stored as a colloid substance within the thyroid follicles. Each Tag molecule has around (100) tyrosine residues, a quarter of which are iodinated. These residues couple to form triiodothyronine T3 and thyroxine T4. When TSH stimulates the thyroid cells, Tag is endocytosing and hydrolyzed in lysosome releasing T3 and T4. The exact location of T- and B- cell epitopes within Tag is uncertain. Carayanniotis and Rao (1997). Thyroglobulin autoantibodies are found in less than (60%) of patients with lymphocytic thyroiditis and (30%) of Graves’ disease patients. They are polyclonal and mainly of IgG class with all four subclasses represented. TSH controls the cell surface expressions of TPO and Tag altering the transcription of these two proteins, possibly at the gene promoter level. These effects are mimicked by blocking and stimulating autoantibodies in sera of patients with Graves’ disease. Collison et al. (1991).

Mild iodine deficiency is associated with lower prevalence of Hashimoto’s disease and hypothyroidism, while excessive iodine intake is associated with a higher prevalence. Gbadebo and Oyesanya (2005). In China, autoimmune thyroiditis was found only in (0.3%) of those having mild iodine deficiency, and (1.3%) of those with excessive iodine intake. Walsh et al. (2006).
2. RATIONALE

There is existent gap in knowledge about autoimmune thyroid disease and its association with thyroid antibodies (anti-thyroid peroxidase and antithyroglobulin). This study was expected to provide sound knowledge for scientist and physician as well for better management of patients with thyroid diseases in Shendi Locality and beyond.

3. OBJECTIVES

The general objective of this research is to evaluate the association between thyroid autoimmune antibodies and thyroid function tests and among patients with nonneoplastic thyroid disease in Shendi Locality.

4. SUBJECTS AND METHODS

The current research was prospective, case-control, hospital-based study carried out from 2013-2017 in Elmek Nimir University hospital in Shendi town-Northern Sudan. River Nile State.

The study included patients with non-neoplastic thyroid diseases attending outpatient clinic Elmek Nimir University hospital in Shendi for routine follow up, as case group. while the control group are healthy subjects without thyroid diseases and match with study group in age and sex distribution.

Random sampling was used to select suitable sample size. Two hundred and eighty-three (283) participants selected as study population; divided into three categories:

1) Control group (healthy); Hundred (100) subjects.
2) Hypothyroidism patients; Hundred and eleven (111) subjects
3) Hyperthyroidism patients; Seventy-two (72) subjects

Structural interview questionnaire was applied to collect the following data: personal data, social customs, food habits, exercise, medical history, weight, height, duration of the disease, type of thyroid drugs.

Three (3) ml of venous blood samples were drawn from every participant in heparinized blood collection tubes, using sterile syringes and centrifuged (1500 r.p.m) for five (5) minutes to obtain heparinized plasma for analysis of thyroid hormones profile (TSH, T4, T3, fT3, and fT4).

Other aliquots of two (2) ml were collected in plain container and were allowed to clot and then centrifuged (1500 r.p.m) for five minutes, the supernatant sera were transferred into a plastic tube (Eppendorf tube) and stored at (-80°C) for the analysis of thyroid antibodies (antithyroid peroxidase, antithyroglobulin antibodies)

TSH, T4, fT4, T3, and fT3, were measured by TOSOH AIA system analyzer, employing standard reagents and procedures; (ST AIA – PACK TS H, Cat. No. 0025294), (ST AIA – PACK T4, Cat. No. 0025258), (ST AIA – PACK FT4, Cat. No. 0025268), (ST AIA – PACK TT3, Cat. No. 0025282), (ST AIA – PACK iFT3, Cat. No.0025231), respectively.

Thyroid antibodies (Anti-thyroid peroxidase) and antithyroglobulin were measured by sequential ELISA method employing (Anti-TPO) ELISA, and (Anti-Tag) ELISA respectively.
5. DATA INTERPRETATION AND ANALYSIS

The collected data was analyzed using Statistical Package for Social Sciences (SPSS) version (11.5). Mean, Standard SD, frequency, percentage, T-test, and correlation were used. Significant level was set at $P \leq (0.05)$.

6. ETHICAL CLEARANCE

The study was firstly approved by ethical committee of the Faculty of Graduate Studies and Scientific Research (Institute Research Board) of Shendi University. Permission was also taken from Elmek Nimr University Hospital administration. Verbal informed consent was taken from all participants Names, personal data were completely secured and transferred to codes so as to keep patients’ identities private, taking into account the trust and strict confidentiality with respect to patients and information about them.

7. RESULTS

| Table 1 Sociodemographic and personal data |
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Positive 51 45.9 22 30.5

**Focal thyroid signs among the study group**

| Focal thyroid signs | Frequency | Percentage % | Frequency | Percentage % |
|---------------------|-----------|--------------|-----------|--------------|
| Solitary nodule     | 21        | 18.9         | 20        | 27.8         |
| Multinodular        | 12        | 10.8         | 7         | 9.7          |
| Diffuse             | 9         | 8.1          | 15        | 20.8         |
| Bruit               | 0         | 0            | 1         | 1.4          |
| No Goiter           | 69        | 62.2         | 29        | 40.3         |

**Other diseases among the study group**

| Other Diseases      | Frequency | Percentage % | Frequency | Percentage % |
|---------------------|-----------|--------------|-----------|--------------|
| Diabetes Mellitus   | 11        | 9.9          | 2         | 2.8          |
| Rheumatoid arthritis| 1         | 0.9          | 1         | 1.4          |
| Pregnancy           | 0         | 0            | 1         | 1.4          |
| Hypertension        | 7         | 6.3          | 2         | 2.8          |

**Table 3 Correlation of TPOAb and thyroid hormones in hyperthyroidism patients**

| TPOAb results | Frequency | Percentage % | Mean of: |
|---------------|-----------|--------------|----------|
|               |           |              | TSH      | T4       | FT4       | T3       | FT3       |
| Negative (<40 IU/ml) | 37       | 51.4         | 1.22     | 135.3    | 28.9      | 2.8      | 7.3       |
| Positive (>40IU/ml)   | 35       | 48.6         | 0.59     | 146.9    | 35.2      | 3.6      | 12.8      |
| P. value           |           |              | 0.122    | 0.488    | 0.3       | 0.147    | 0.032*    |

**Table 4 Correlation of thyroid peroxidase antibodies and thyroid hormones in hypothyroidism patients**

| TPOAb results | Frequency | Percentage % | Mean of: |
|---------------|-----------|--------------|----------|
|               |           |              | TSH      | T4       | FT4       | T3       | FT3       |
| Negative (<40 IU/ml) | 39       | 35.1         | 18.5     | 63.6     | 12.5      | 1.36     | 3.3       |
| Positive (>40IU/ml)   | 72       | 64.9         | 22.7     | 63.6     | 11.8      | 1.35     | 3.7       |
| P. value           |           |              | 0.561    | 0.999    | 0.513     | 0.874    | 0.029*    |

**Table 5 Correlation of thyroglobulin antibodies and thyroid hormones in hyperthyroidism patients**

| TgAb Results      | Frequency | Percentage % | Mean of: |
|-------------------|-----------|--------------|----------|
|                   |           |              | TSH      | T4       | FT4       | T3       | FT3       |
| Negative (<125 IU/ml) | 50       | 69.5         | 0.95     | 129.1    | 28.1      | 2.7      | 7.7       |
| Positive (>125IU/ml) | 22       | 30.5         | 0.82     | 167.8    | 40.8      | 4.2      | 15.4      |
| P. value           |           |              | 0.776    | 0.030*   | 0.048*    | 0.014*   | 0.004**   |

**Table 6 Correlation of thyroglobulin Antibodies and thyroid hormones in hypothyroidism patients**

| TgAb Results      | Frequency | Percentage % | Mean of: |
|-------------------|-----------|--------------|----------|
|                   |           |              | TSH      | T4       | FT4       | T3       | FT3       |
Table 7 Correlation between serum thyroid hormones in case and control groups

| Thyroid Hormone | Hypothyroidism | Hyperthyroidism |
|-----------------|---------------|-----------------|
| N=111           | N=72          |                 |
| TSH             |               |                 |
| Group           | Frequency     | Mean            | Sig. (2-tailed) | Frequency | Mean            | Sig. (2-tailed) |
| Case            | 111           | 21.19           | 0.000**        | 72        | 0.9             | 0.000**        |
| Control         | 100           | 2.1             |                | 100       | 2.1             |                |
| TT4             |               |                 |                 |           |                 |                 |
| Case            | 111           | 63.6            | 0.000**        | 72        | 140.9           | 0.000**        |
| Control         | 100           | 93.1            |                | 100       | 93.1            |                |
| FT4             |               |                 |                 |           |                 |                 |
| Case            | 111           | 12.1            | 0.000**        | 72        | 31.97           | 0.000**        |
| Control         | 100           | 17.6            |                | 100       | 17.6            |                |
| TT3             |               |                 |                 |           |                 |                 |
| Case            | 111           | 1.17            | 0.000**        | 72        | 3.36            | 0.000**        |
| Control         | 100           | 1.58            |                | 100       | 1.58            |                |
| FT3             |               |                 |                 |           |                 |                 |
| Case            | 111           | 3.58            | 0.000**        | 72        | 10.04           | 0.000**        |
| Control         | 100           | 4.26            |                | 100       | 4.26            |                |

8. DISCUSSION

The population of this study were distributed according to occurrence of thyroid disease into (111) participants (60.7%) with hypothyroidism, and: (72) participant (39.3%) with hyperthyroidism, then into sex according to disease as follows: (91.9%) of hypothyroidism were females and just (8.1%) were males, in hyperthyroidism; (84.7%) were females and (15.3%) were males.

Family history: (33.3%) of hypothyroidism were with family history, (64.9%) of them with first degree and (35.1%) with second degree of family history and (66.7%) without a family history, in hyperthyroidism; (37.5%) were with positive family history (70.4%) of them with first degree and (29.6%) with second degree and (62.5%) without family history. (50.5%) of hypothyroidism and (41.7%) of hyperthyroidism patients were newly discovered. The age in hypothyroidism; the mean of age was (50.4± 14.7 years), in hyperthyroidism was (43.6±13.4 years). The body weight in hypothyroidism was (68.1±15.5 Kg), and in hyperthyroidism was (64.9±13.5kg). The mean level of TSH in hypothyroidism was significant increased than control group (P. value=0.000), that means there was highly significant statistical different between the means, and it is out of range (0.4 – 4.3 µIU/mL), the mean in hyperthyroidism had statistically significant difference (P. value=0.000) but within reference range. There were highly significant statistically differences between the mean of thyroid parameters, in hypothyroidism, control and hyperthyroidism group (P. value=0.000). The results of this study were consistent with study conducted in China by Hong Li, et al., indicating that the correlation of TT4, and fT4 with TSH was statistically significant in healthy individuals (P < 0.01).
The correlation of TT4, fT4, TT3, and fT3 with TSH was statistically significant in patients with hyperthyroidism. The correlation of TT4, fT4, TT3, and fT3 with TSH was statistically significant in patients with hypothyroidism. TSH and fT4 are the most valuable indicators in assessing thyroid function in a healthy population, and TSH and TT4 are the most meaningful in hyperthyroidism and hypothyroidism. The current study showed that: 107 (58.5%) patients were positive when evaluated to TPO Ab with level more than (40.0 IU/ml), 72 (64.9%) of hypothyroidism group were TPO Ab positive and 35 (48.6%) of hyperthyroidism have positive titter of TPO Ab, in evaluation of Tag Ab, 73 (39.9%) of the population studied were positive, 51 (69.9%) of them were hypothyroidism, and 22 (30.1%) of them were hyperthyroidism. Regarding the correlation between TPO Ab and Tag Ab in hypothyroidism the study findings were in agreement with a study found that: the TPO Ab positive patients, (60.7%) were found to be hypothyroid and TagAb positive patients; (53.1%) patients were hypothyroid, while the correlation between thyroid antibodies and hyperthyroidism appear to be. also similar to the findings of the study conducted in Iran by Aminorroaya et al. (2006) who found that; positive autoantibodies were detected in (75.5%) of patients with hypothyroidism, and in (73.6%) of those with hyperthyroidism.

In hyperthyroidism patients, the mean level of fT3 with positive TPO Ab was statistically increased more than the negative TPO Ab (P. value= 0.032) that means there was statistically significant effect of presence of TPO Ab on serum level of fT3.

9. CONCLUSION

- Most of thyroid patients were with hypothyroidism.
- Most thyroid dysfunctions were females.
- Free fractions of thyroid hormones have more correlations with clinical findings.
- (58.5%) of thyroid patients had TPO Ab, (39.9%) had Tag Ab.
- Two third of hypothyroidisms had positive titter of TPO Ab and Tag Ab.
- One half of hyperthyroidisms had TPO Ab positive titter and one third had Tag Ab.

10. RECOMMENDATIONS

   Based on the results, obtained from this study, it is recommended that:
   1) Evaluation of thyroid antibodies must be done for all thyroid patients.
   2) As concern thyroid hormones evaluation, free fraction, total thyroxine, and measurement of binding protein also important to distinguish between thyroid dysfunction and deficiency of binding proteins.
   3) Further studies should be done for elucidation of genetic and environmental factors triggering production of autoantibodies.
   4) Presence of thyroid antibodies should be taken in consideration with hormonal assays to give precise diagnosis of thyroid disease.

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