HISTOLOGICAL PATTERN IN IMMUNOLOGICAL THYROID DISEASE PATIENTS AT MOI TEACHING AND REFERRAL HOSPITAL (MTRH), WESTERN KENYA

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ABSTRACT

Problem statement: There are many thyroid pathologies in western Kenya but their morphological changes have not been reported in autoimmune thyroid disorders.

Study population: Three hundred and eighty eight patients were studied between Jan 2008 to Dec 2011.

Objectives: To describe the histological patterns in immunological thyroid diseases patients at MTRH, western Kenya.

Methodology: 388 patients with thyroid pathologies had thyroidectomy and histological diagnosis done. Under light microscopy, sections were examined to determine whether thyroid follicles were present in the tissues. The retrospectively collected data was analyzed using STATA version 10 SE. The results were presented in form of plates, tables, and charts.

Results: Among the histological diseases, colloid goitre was the most prevalent in all the age groups. Across the age groups, ages 30-39 & 40-49 bore the greatest burden of thyroiditis, thyroid carcinoma, thyroid adenoma and colloid goitre. Similarly, colloid goitre was the most prevalent among the male as well as among the female patients. The median age of the subjects was 40 (33-50). There was no significant difference (p=0.154) in age between the males and the females. Conclusion: Histological examination of the thyroid gland combined with measurement of plasma thyroid autoantibodies levels could be used for evaluation of immunological thyroid disease. The prevalence of immunological thyroid disease is higher than the reported prevalence in Kenya.

Key words: Thyroid cyst, thyroid adenoma, Hashimoto thyroiditis, colloid goitre, thyroid carcinoma, thyroid abscess.
Introduction

The principal diseases of the human thyroid gland are hyperthyroidism, hypothyroidism, autoimmune thyroiditis and neoplasms [1, 2]. The human autoimmune thyroid disorders (AITDs) broadly includes Grave disease (GD) and Hashimoto’s thyroiditis (HT) which are the most common causes of thyroid gland dysfunctions and non endemic goiter[3]. These conditions arise due to complex interactions between environmental and genetic factors [4, 5, 6] and are characterized by reactivity to self-thyroid antigens which are expressed as distinctive inflammatory or antireceptor autoimmune diseases [7, 8, 3]. The first pathological features of autoimmune thyroiditis were described in 1922 [9]. Hashimoto’s. The goiter exhibits lymphocytic infiltrate with prominent germinal centers, presence of Hurthle cells, and areas of fibrosis. The antibodies are directed against thyroid antigens and are the most common cause of hypothyroidism. The incidence is 0.3 to 1.5 per 1000 person per years, and it is 4 to 10 times more common in women than in men [10, 4].

Graves’ disease on the other hand, involves the binding of autoantibodies to TSH receptor which lead to stimulation. It is the most common cause of thyrotoxicosis with female-to-male ratio is 3.5:1 [11]. Graves’ disease features include, swelling over the anterior shin (pretibial myxedema), thyroid eye disease (prominence of eyes, lid lag, globe lag, exophthalmos, lid edema, chemosis, and extraocular muscle weakness); and increased pigmentation and vitiligo. Thyroid ophthalmopathy is present in about 50% of Graves’ patients [12].

Autoimmune thyroid diseases have been reported in people living in different parts of the world including North America, Europe, Baalkans, Asia, Middle East, South America and Africa. The reported figures however do not fully reflect the number of people infected per year. Cases are unrecognized due to inaccurate diagnosis and hence are treated as other diseases. Its prevalence in Egypt is 1,522,348 while South Africa has a prevalence of 888,969 The annual incidence of Hashimoto’s thyroiditis worldwide is estimated to be 0.3-1.5 cases per 1000 persons whereas Grave’s disease is estimated at about 5 per 10,000 people [3, 13]. However, the most recent studies have shown that the human autoimmune thyroid diseases (AITDs) affect up to 5% of the general population [5, 10].

In Kenya, the available reports show that the extrapolated prevalence of autoimmune thyroid disease is 659, 642 for an estimated population of 32, 982, which ideally is for studies that were carried out between 1974 and 1978 [14, 15]. This indicates that there is paucity of data on the prevalence and etiology of the human autoimmune thyroid diseases. The present study was therefore designed to describe the histological changes in immunological thyroid diseases patients at MTRH, western Kenya.

Material and Methods

Study site

This research was carried out at Moi Teaching and Referral Hospital-Eldoret. This is a hospital that serves clients from all over North-Rift, parts of western Kenya and Nyanza province.

Study design

This study was retrospective survey design in which all patients with thyroid pathologies and who underwent thyroidectomy at the MTRH between 2008 and 2011 were included. Stored tissues were retrieved and processed for immunohistochemical studies by detection of the thyroid autoantibodies directed against thyroid antigen.

Methodology

Data on tissue histopathology diagnosis was collected through the use of pathology reports in the histopathology laboratory. The histology report laboratory number was used to retrieve the tissue blocks.

Histology

All thyroid specimen collected during thyroidectomy were then transferred to histology department of MTRH for histological processing. The tissues were formalin fixed, paraffin embedded sections sliced using a microtome and slices were mounted on slides .The slices were then stained with Haematoxilin and Eosin (H&E). The slides were then microscopically examined for the presence of histological disease using a
microscope. Each slide was first examined entirely at x5 and x40 objective. As the examination was going on, the slides were photographed at the same magnification using a Kodak camera mounted on the same microscope. However, the observations made were recorded in terms of absence or presence of the thyroid disease. Also histological feature of each thyroid pathology examined were recorded. Each slide was then dipped into xylene after every examination to remove the oil immersion from its surface and stored in a slide box for future reference.

**Immunohistochemistry**

About 4-40 um thick of formalin and paraffin embedded sections were sliced using a microtome and slices were mounted on slides. The slides were then taken for immunohistochemistry processing in which the antigen (centromere, SS-A(Ro), Scl-70 and PCNA/Cyclin ) was localized in cells of the thyroid tissue section exploiting the principle of antibody specifically to antigens in biological tissues. Visualisation of antibody antigen reaction was accomplished through an antibody (IgG) conjugated to FITC anti-human globulin that catalyzed a color (apple green) producing reaction which indicated a positive reaction when viewed under fluorescence microscope. Clinical data was obtained from the hospital record files for purposes of documenting the disease trends over the past four years (2008-2011).

**Determination of the prevalence of immunological thyroid diseases**

Patients with thyroid pathologies who were not on any immunosuppressant drugs and underwent thyroidectomy at the MTRH between 2008 and 2011 were recruited. Prevalence was calculated as the proportion of patients with thyroid disease divided by the total number of patients examined for immunological thyroid disease expressed as a percentage. The corresponding 95% confidence limits were calculated. The number of patients with a particular thyroid disease and the prevalence value were tabulated. The prevalence was computed, stratified by gender and for combined groups.

**Ethical considerations**

Institution review ethics committee (IREC) approval was obtained before starting data collection. Findings were discussed with the relevant health provider. Information was provided in appropriately accessible language.

**Statistical Analysis**

Data analysis of four years (2008-2011) biopsy material obtained from patients was done using STATA version 10 SE (College Station, Texas, USA). Categorical variables were summarized as frequencies (percentage) while the continous variables were summarized as median (quartiles). Non-parametric tests Wilcoxon rank sum test used to compare the distributions of continuous variables between any two independent groups. Age was categorized at the median and separately at ten year intervals. The comparison of the proportions of two independent groups was done using the two sample z-test. The prevalence levels were compared with the national and regional data.

**Results**

There were 388 subjects aged between 14 and 89 years who were eligible for analysis and were categorized as having been diagnosed with thyroid gland disorders, autoimmune thyroid disease or not. Histological pictures were used to classify the conditions as thyroiditis or not, thyroid carcinoma, thyroid adenoma, colloid goitre, thyroid cysts or thyroid abscess (Plates; 1a, 1b, 1c, 1d, and 1e). The frequencies and the corresponding percentages are presented in Tables 1a and 1b.

**Histological patterns in immunological thyroid diseases**

Below are some of the images of thyroid histology taken during the study and their description.
Plate (1a) Hashimoto thyroiditis: The tissues shows late stage Hashimoto thyroiditis with thyroid architecture distorted by fibrosis with lymphoid follicles containing reactive germinal centre (lymphocytes, plasma cells and occasional multinuclear giant cells) under x25 and x40 magnification.

Plate (1b) Colloid goiter: shows colloid goiter with large and small follicles filled with colloid that appears quiescent. Abundant colloid is seen in the hugely distended follicles when viewed under x25 and x40 magnification.

Plate (1c) papillary carcinoma: This tissue section shows scar-like fibrotic area which may intercept neoplastic glands. The fibrous brands may extend outward and divide the tumor into large clusters. Finger-like
projections lined by neoplastic cells when viewed under x25 and x40 magnification. In normal condition, thyroid gland contains numerous follicles, composed of epithelial follicle cells and colloid. Also, between follicles are clear parafollicular cells, which produce calcitonin (hormone for calcium balance).

**Plate (1d) Follicular adenoma:** shows small follicles contained in the adenoma. The normal thyroid has been compressed with much larger follicles at the bottom than those within the adenoma. There is no invasion of the capsule when viewed under x25 and x40 magnification.

**Plate (1e) Follicular carcinoma:** The tissue section show vascular invasion in follicular carcinoma with tumor cells invading a capsular vessel. The vascular invasion seen here is evidence for malignancy and hence there is possibility of metastasizing to other tissues. The features of papillary carcinoma are lacking, so this is a follicular carcinoma composed of cells that are not highly pleomorphic or hyperchromatic. It can be difficult to tell a follicular carcinoma from an adenoma by histological appearance alone, and the term "follicular neoplasm" may be utilized. Follicular carcinoma, the second most common thyroid malignancy, tends to be indolent.
Table 1a: Distribution of histological diseases

<table>
<thead>
<tr>
<th>Histological diseases</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroiditis</td>
<td>24</td>
<td>(7.2)</td>
</tr>
<tr>
<td>Thyroid Carcinoma</td>
<td>18</td>
<td>(4.6)</td>
</tr>
<tr>
<td>Thyroid Adenoma</td>
<td>51</td>
<td>(13.1)</td>
</tr>
<tr>
<td>Colloid Goitre</td>
<td>286</td>
<td>(73.7)</td>
</tr>
<tr>
<td>Thyroid Cyst</td>
<td>8</td>
<td>(2.1)</td>
</tr>
<tr>
<td>Thyroid Abscess</td>
<td>1</td>
<td>(0.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>388</strong></td>
<td><strong>(100)</strong></td>
</tr>
</tbody>
</table>

There were 24 (7.2%; 95% confidence limits (CL): 4.0-9.1) subjects who had the autoimmune thyroid disease with subjects aged above the median of 40 (IQR: 33-50) having a non significant higher rate (p-value=0.654) of autoimmune thyroid disease (6.4%; 96% CL: 3.0-10.0) compared to those aged below the median (5.4%; 95% CL: 2.0-8.9). Table 1b shows autoimmune thyroid diseases (thyroiditis types) and their corresponding percentages.

Table 1b: Patterns of autoimmune thyroid diseases

<table>
<thead>
<tr>
<th>Autoimmune thyroid diseases (thyroiditis)</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulomatous thyroiditis</td>
<td>1</td>
<td>(4.17)</td>
</tr>
<tr>
<td>Grave’s thyroiditis</td>
<td>4</td>
<td>(16.67)</td>
</tr>
<tr>
<td>Hashimoto thyroiditis</td>
<td>18</td>
<td>(75.00)</td>
</tr>
<tr>
<td>Unspecified Thyroiditis</td>
<td>1</td>
<td>(4.17)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>24</strong></td>
<td><strong>(100)</strong></td>
</tr>
</tbody>
</table>

The sample was mainly made up of female 368 (95%). There was no case of autoimmune thyroid disease that was reported among the male patients. From Table 1a, colloid goiter was the most prevalent of all the histological diseases. Thyroid abscess was seen in one patient only.

The histological disease patterns stratified by age groups and by sex are given in Tables 2 and 3, respectively. From the results, colloid goitre among all the histological diseases presented itself with the greatest proportion in all the age groups. Across the age groups, ages 30-39 & 40-49 bore the greatest burden of thyroiditis, thyroid carcinoma, thyroid adenoma and colloid goitre. Similarly, colloid goitre was the most prevalent among the male as well as among the female patients. Due to small number of the male patients, interpreting the distribution of the histological diseases across gender will not be statistically sound. However, we have given the proportions across gender.
Table 2: Distribution of immunological thyroid diseases by age categories

<table>
<thead>
<tr>
<th>Age</th>
<th>Thyroiditis</th>
<th>Thyroid Carcinoma</th>
<th>Thyroid Adenoma</th>
<th>Colloid Goitre</th>
<th>Thyroid Cyst</th>
<th>Thyroid Abscess</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>0 (0; 0)</td>
<td>1 (14.3; 5.6)</td>
<td>0 (0; 0)</td>
<td>5 (71.4; 1.8)</td>
<td>1 (14.3; 12.5)</td>
<td>0 (0; 0)</td>
<td>7 (100; 1.8)</td>
</tr>
<tr>
<td>20-29</td>
<td>3 (7.1; 12.5)</td>
<td>0 (0; 0)</td>
<td>7 (16.7; 13.7)</td>
<td>32 (76.2; 11.2)</td>
<td>0 (0; 0)</td>
<td>0 (0; 0)</td>
<td>42 (100; 10.8)</td>
</tr>
<tr>
<td>30-39</td>
<td>6 (5.1; 25)</td>
<td>2 (1.7; 11.1)</td>
<td>17 (14.5; 33.3)</td>
<td>91 (77.8; 31.8)</td>
<td>1 (0.9; 12.5)</td>
<td>0 (0; 0)</td>
<td>117 (100; 30.2)</td>
</tr>
<tr>
<td>40-49</td>
<td>8 (7.8; 33.3)</td>
<td>8 (7.8; 44.4)</td>
<td>16 (15.7; 31.4)</td>
<td>69 (67.7; 24.1)</td>
<td>1 (1.0; 12.5)</td>
<td>0 (0; 0)</td>
<td>102 (100; 26.3)</td>
</tr>
<tr>
<td>50-59</td>
<td>2 (4.1; 8.3)</td>
<td>1 (2.0; 5.6)</td>
<td>3 (6.1; 5.9)</td>
<td>41 (83.7; 14.3)</td>
<td>2 (4.1; 25)</td>
<td>0 (0; 0)</td>
<td>49 (100; 12.6)</td>
</tr>
<tr>
<td>60-69</td>
<td>2 (8.7; 8.3)</td>
<td>0 (0; 0)</td>
<td>4 (17.4; 7.8)</td>
<td>16 (69.6; 5.6)</td>
<td>0 (0; 0)</td>
<td>1 (4.4; 100)</td>
<td>23 (100; 5.9)</td>
</tr>
<tr>
<td>70 and over</td>
<td>1 (3.5; 4.2)</td>
<td>5 (17.2; 27.8)</td>
<td>2 (6.9; 3.9)</td>
<td>20 (69.0; 7.0)</td>
<td>1 (3.5; 12.5)</td>
<td>0 (0; 0)</td>
<td>19 (100; 7.5)</td>
</tr>
<tr>
<td>Adults</td>
<td>2 (10.5; 8.3)</td>
<td>1 (5.3; 5.6)</td>
<td>2 (10.5; 3.9)</td>
<td>12 (63.2; 4.2)</td>
<td>2 (10.5; 25.0)</td>
<td>0 (0; 0)</td>
<td>19 (100; 4.9)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (6.2; 100)</td>
<td>18 (4.6; 100)</td>
<td>51 (13.1; 100)</td>
<td>286 (73.7; 100)</td>
<td>8 (2.1; 100)</td>
<td>1 (0.3; 100)</td>
<td>388 (100; 100)</td>
</tr>
</tbody>
</table>

Adults are patients who did not have the age given but were indicated as the adults.

Table 3: Distribution of immunological thyroid diseases by sex

<table>
<thead>
<tr>
<th>SEX</th>
<th>Thyroiditis</th>
<th>Thyroid Carcinoma</th>
<th>Thyroid Adenoma</th>
<th>Colloid Goitre</th>
<th>Thyroid Cyst</th>
<th>Thyroid Abscess</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>24 (6.5; 100)</td>
<td>16 (4.4; 88.9)</td>
<td>49 (13.3; 98.1)</td>
<td>271 (73.6; 94.8)</td>
<td>7 (1.9; 87.5)</td>
<td>1 (0.3; 100)</td>
<td>368 (100; 95.0)</td>
</tr>
<tr>
<td>Male</td>
<td>0 (0; 0)</td>
<td>2 (10.0; 11.1)</td>
<td>2 (10.0; 3.9)</td>
<td>15 (75.0; 5.2)</td>
<td>1 (5.0; 12.5)</td>
<td>0 (0; 0)</td>
<td>20 (100; 5.0)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (6.2; 100)</td>
<td>8 (4.6; 100)</td>
<td>51 (13.1; 100)</td>
<td>286 (73.7; 100)</td>
<td>8 (2.1; 100)</td>
<td>1 (0.3; 100)</td>
<td>388 (100; 100)</td>
</tr>
</tbody>
</table>

The median age of the subjects was 40 (33-50). Stratified by gender, there was no significant difference with regards to age distribution: male 52 (37-73) and female 40 (33-50) with p-value=0.154.

In plate 2(a) and 2(b) show thyroid sections with autoantibodies staining bright apple green fluorescence (arrows) when incubated with anti-human globulin IgG conjugated to fluorescein isothiocyanate (FITC). Patterns of staining can vary from nuclear and/or cytoplasmic staining and can be exhibited depending on the types and relative amounts of autoantibodies present in the tissue section.

Below are thyroid tissues from a patient with colloid goiter showing dense deposits of IgG along the basement membrane of follicular cells under high power microscopy (fluorescent microscope) at X 40 magnification.
The proportions of colloid goiter among those patients who did not have immunological thyroid disease (not immunological) was higher than in the patients in the immunological group while the proportion of subjects suffering from thyroid adenoma was higher in the immunological group (group that were positive when tested with anti-human globulin IgG = 213 subjects) compared to the non-immunological group (group that were negative when tested with anti-human globulin IgG = 175 subjects). However, the rest of the histological diseases showed no visible difference in their proportions between the two groups except for the subject suffering from thyroid abscess who was non-immunological, see Figure 1.

Figure 1: Proportion of histological thyroid diseases stratified by immunological status
Discussion

In summary, this study has shown that various thyroid diseases express thyroid autoantibodies. However the pattern of expression was different when immunological and non-immunological thyroid diseases were compared. Only in immunological thyroid disease group had a significant expression of autoantibodies.

The study population was mainly made up of females 368 (95%) implying that the disease is more prevalent in females and this is in agreement with other studies [16]. There was no case of autoimmune thyroid disease among the male patients. However, this may be attributed to the fact that there were very few male subjects compared to females.

Colloid goiter represented the largest proportion of the histological diseases as it was seen in 286 (73.7%) of the cases in this study. This prevalence is higher than the earlier reported prevalence (47.7%) in a retrospective histopathological study done on 575 histopathological reports over a nine year period in Kenya [14]. This might be due to the difference in indications for the thyroidectomy and availability of histopathological laboratories. The difference could also be due to a difference in the magnitude of the disease. That is how long one has stayed with the disease and the extent of damage caused by the disease. Colloid goiter is most common throughout the world and most prevalent in mountainous regions [17]. This is due to fact that there is lack of iodine in these regions [3]. A deficient intake of iodine is the dominant cause of colloid goiter. Lack of iodine leads to decrease in the synthesis of thyroid hormones with a compensatory increase in secretion of thyroid stimulating hormone which in turn may lead to follicular cell hypertrophy and hyperplasia and after sometime enlargement of goiter occurs. Most of the time the enlarged goiter is diffuse in nature but may take the form of discrete nodules. This should be distinguished from enlargement of the gland due to malignancy. The enlargement of thyroid gland can be viewed as homeostatic mechanism to maintain thyroid hormone levels [18]. It occurs in response to elevated concentrations of thyrotropic hormones. If this compensation is successful, adequate thyroid hormone levels will be maintained and the person will be euthyroid. In some instances, hypothyroidism may be associated with goiter. Even euthyroid goiters can result in specific problems especially if they are so large as to constrict the neck and interfere with breathing and eating. Other predisposing factors include the presence of anti thyroid substance in the diet, drugs, mineral and bacterial contamination of the drinking water [19]). These factors block the synthesis of thyroid hormone which leads to the enlargement of goiter.

The prevalence of adenomas in this study was 51 (13.1%). It represented the second largest proportion of the histological diseases. This prevalence was consistent with the study made by Namba (1976), Gitau (1975) but lower than report made by Kungu (1974). The age and sex distribution of adenoma in this study, increased with age increases. This was consistent with other studies [16].

Thyroid follicular carcinomas presenting with 18 (4.6%) were seen in this study. This prevalence rate was lower than the 23.3% cases reported by Kungu (1974). Carcinoma of the thyroid are uncommon and mostly in adults although some form may occur in childhood such as papillary thyroid carcinoma [16]. Female are more predisposed than the males and was noted among patients presenting with thyroid carcinoma in the middle adult age. The sex and age distribution of thyroid carcinoma seen in this study was consistent with other studies [16].

This prevalence rate of immunological thyroid diseases seen in this study is higher (7.2%) than the one reported by Kungu (3%) and Gitau (1%) [14, 15].The median age of the subjects was 40 (33-50). Stratified by gender, there was no significant difference with regards to age distribution: male 52 (37-73) and female 40 (33-50) with p-value=0.154.

The other histological thyroid disease encountered in this study was thyroiditis 24 (7.2%). This high prevalent rate is higher than the reported prevalence 3% [14] and 1% [15]. The study sample was made up of 368 (95%) female and out of this, 24 (7.2%) were females. There was no case of thyroiditis in males.

Thyroid abscess is a rare condition of the thyroid gland. In this study, the prevalence of thyroid abscess was 0.3%. This presented the least of thyroid pathologies seen in this study. The ability of thyroid gland is to
resist infection which is well known and the infection in thyroid gland is rare, particularly so with the advent of widespread usage of antibiotics. Thyroid abscess represents only 0.1 to 0.7% of surgically treated thyroid pathologies [18].

The other thyroid pathology found in this study was thyroid cyst. Thyroid cyst presented with the prevalence rate of 8 (2.1%). The prevalence of thyroid cyst has not been reported by other studies in Kenya. The cystic portion of thyroid is considered to be caused by hemorrhage and subsequent degeneration of preexisting nodules [20]. The cystic changes in metastatic lymph nodes occur in certain types of tumors, site-specific phenomenon that mostly happens in the lymph nodes of head and neck region. Although other thyroid diseases like hyperthyroidism and thyrotoxicosis were not described in this study, it does not mean they are uncommon [3].

Conclusions

This study concludes that thyroid pathologies are present in Kenya. The prevalence of autoimmune thyroid disease was higher (7.2%) than the reported by other studies (2%) in Kenya and Africa at large. The prevalence was higher in females (94%) than in males (5.9%). Among the histological types of thyroid disease goiter presented with the highest prevalence (73.7%) and that it is the most common histological disease. Studies have also proved that colloid goiter is as a result of iodine deficiency and iodine supplement was found to decrease the prevalence of goiter. Hence this study recommends that iodine supplement to be effected to reduce the prevalence of colloid goiter. This will help reduce the prevalence of this disease. This will help to reduce the resources spent on managing individual goiter cases besides alleviating the social impact of goiter. Histological examination of the thyroid gland combined with measurement of plasma thyroid autoantibodies levels could be used for evaluation of immunological thyroid disease.

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Reference


