Microbial Etiology to Thyroid Pathology

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Abstract

Introduction
Microbial infections are normally cited as a main environmental factor involved in pathology. The current study aimed at determining frequency of occurrence of pathogens in thyroid pathology. Our study is focused on human data. We have reviewed thyroid Fine Needle Aspiration (FNA) samples for pathogens.

Methods
Biopsy by fine-needle aspiration was applied in investigating the nodule for microbial involvement. Two smears were prepared on slides from each sample. The first slide was processed through Ziehl Neelsen. The second slide was processed in gram stain, for the identification and classification of bacteria.

Results
Of the total 41 sampled patients, evaluation of the presence of microorganisms in the thyroid FNA biopsies based on ZN and gram stains showed that a minimal percentage (12%) of the patients had a microbial aetiology. Among them, 2 cases (4.8%) had acid fast bacilli. Further 3 cases (7.2%) had gram negative rods.

Conclusion
The study findings suggested that the thyroid is less likely to suffer microbial infections. This is majorly due to the rich supply of the thyroid with both blood and lymphatic fluid and the presence of iodine which harbours some bactericidal properties.

Keywords: Thyroid pathology, Ziehl Neelsen, gram stain

1. Introduction
Thyroid gland is a double-lobed organ located at the neck region. In some cases pathological nodules surround the thyroid causing changes in the size and shape of the organ (1). Bacterial infection may cause fluid-filled cavities in the thyroid commonly known as thyroid cyst. These conditions most commonly will result from the activities of such bacteria in the breakdown of cells and consequently degenerating thyroid tissue (2). Any of the wide range of bacteria would infect the thyroid including but not limited to *Streptococcus spp*, *Staphylococcus aureus*, *Salmonella* and *Klebsiella* species (3). *Candida albicans*, *Aspergillus* and *Actinomycetes* are among the fungus infecting the thyroid (3).

The development of thyroid nodules has also been found to be linked with the infection of individuals with tuberculosis mostly in the sub-Saharan African. In a study conducted in Somali, it indicated the occurrence of thyroid tuberculosis in an individual who was euthyroid but presenting with a thyroid mass (4). Tuberculosis can invade most of the body tissues and the Ziehl Neelsen (ZN) stain is a special bacteriological stain that is important for the identification of acid-fast organisms. Acid fast bacteria will include but not limited to *Mycobacterium tuberculosis* that is responsible for tuberculosis (TB).

In the diagnosis of thyroid conditions, an invasive procedure known as a fine needle aspiration biopsy (FNAB) is conducted to collect cell sample. This procedure is conducted to a patient under local anaesthesia and in few cases will not require anaesthesia (5). Once the sample is collected, it is immediately smeared on a microscope slide and fixed more preferable with a spray fixative. Fixation is followed by staining under an appropriate staining technique based on the investigation being conducted.

Gram staining can be conducted on the prepared slide. It is an important step in the identification and classification of bacteria. It differentiates bacteria into two large categories that are gram positive and gram negative (6). This technique will involve application of a primary stain that is crystal violet on to the fixed slide followed by an iodine...
solution. Iodine serves to bind the primary stain and make it remain bound to the cells. Alcohol is then applied in a step referred to as decolorization; here either ethanol or acetone can be used. The final stage is the application of a counter stain to create contrast; Safranin or Carbol fuchsin can be applied as a counter stain.

Ziehl Neelsen staining (Zn stain) is another example of staining technique that can be applied on the thyroid sample. This staining procedure is used to stain acid fast bacilli. In the thyroid gland it is applied to identify cases of invasive TB(7). These bacteria stain with primary stain which contains a red dye and they retain the dye when treated with acid, which is because of the presence of mycolic acid in their cell wall (7). The reagents used in the process include; Carbol fuchsin which is a basic dye, sulphuric acid which acts as the decolorizer and Methylene blue which is the counter stain (8). The procedure involves Fixing the smear of the specimen over the glass slide, either by heating or alcohol fixation then carbol fuchsin is poured over the smear and heated gently until fumes appear then it is washed off with water. Sulphuric acid is added and allowed to stand for one minute and keep on repeating this step until the slide appears light pink in colour which is then washed off with water. Methylene blue is finally added for two minutes and again washed with water. The slide is allowed to air dry and examined under oil immersion lens. Acid fast bacilli will stain pink, they are straight or slightly curved rods, at times having beaded appearance. The background appears blue due to methylene blue (7).

2. Methods

Biopsy by fine-needle aspiration was applied in investigating the nodule for microbial involvement. The success of a biopsy was dependent on the adequacy of the sample collected and the skill brought about by the experience of the cytopathologist performing the FNA. The procedure did not require the patients to be under local anaesthesia, this was after consulting other research done (9). During the procedure, discomfort was kept mild as the specimens were taken from several parts of the nodule.

Two smears were prepared on slides from each sample. The first slide was processed through Ziehl Neelsen (ZN) staining, this stain is important for the identification of acid-fast organisms (10). The second slide was processed in gram stain, this is an important step for the identification and classification of bacteria (11). Gram staining differentiated bacteria into two large categories that is, the gram positives and gram negative.

3. Results

Evaluation of the presence of microorganisms in the thyroid FNA biopsies (based on ZN and gram stains) showed that 12% of the patients had a microbial aetiology. Among the cases showing microbial involvement, 2 cases (4.8%) had acid fast (ZN stain) bacilli (Figure 2). Furthermore 3 cases (7.2%) had gram negative (gram stain) rods (Figure 2).

The results are shown as proportions of subjects (%), with the blue region representing proportions of individuals without bacterial infection and red region showing proportion with bacterial infection.

Figure 1: Prevalence of Microorganisms in Thyroid FNA Smears

Figure 2: Type of Microorganism Presenting in Thyroid FNA Smears
The results are shown as numbers and proportions of subjects (%), with the bars representing proportions of individuals with gram negative rods and acid-fast bacilli, respectively.

4. Discussion

Only 12.2% of the total FNAs were infected with bacteria. This low level of infection suggests dissemination from other body systems. A previous study found out that the thyroid gland is in some way resistant to infection. This could be explained by the fact that the gland has rich supply of blood and also has an excellent lymphatic drainage. High levels of free iodine content also provide some bactericidal effects. With these two studies finding conclusion was arrived at that the low percentage of thyroid bacteria was due to unfavourable conditions within the thyroid for microbial growth.

Among the 12.2% of FNAs positive for bacteria, three cases had gram negative bacteria. Contrary to this study finding, the most common pathogen that affects the thyroid gland has been thought to be a gram positive Staphylococcus aureus. Staphylococci can be transmitted through inhaling infected air or through the blood stream. Two other cases presented positive for acid fast bacilli. Previous research has shown that TB in the thyroid is a rare occurrence. In cases of thyroidal TB, infection is either through primary infection or secondary infection. Primary infection is when the bacilli directly infect the gland while secondary infection is when the bacilli move to the thyroid from other infected organs.

5. Conclusion

The study findings suggested that due to the rich supply of the thyroid with both blood and lymphatic fluid and the presence of iodine which harbours some bactericidal properties, the thyroid is less likely to suffer microbial infections. Despite this finding, it is in order to stain thyroid samples for infection to point out the few probable cases. This will help in the proper management of such patients and reduce the cost of performing other test that will lead to a waste of resources. The identification of the particular microorganisms will also be important in advising on the proper regime of treatment to be administered.

6. Conflict of Interest and Source of Funding

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7. Authors Contribution

ENand TW designed the study. EN performed slide staining, microbial assessment and reporting. TWand DM did data interpretation. EN and TW co-drafted the manuscript. All authors read through and approved the manuscript.

8. Availability of Data and Material

These data have not been made publicly available as these concerns patient related date which has otherwise been held confidential.

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