

Cellular Patterns of Sudanese Thyroid Lumps in Papanicolaou and Feulgen Stained Fine Needle Aspirates

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Abstract:

Background: Fine needle aspiration (FNA) represent an emerging method for diagnosis of various lump and in particular thyroid lesions. However, there are different techniques to modulate specific cellular pattern of the lesions. Therefore, the aim of this study was to assess the cellular pattern of thyroid lumps in Papanicolaou and Feulgen stained Fine Needle Aspirates. **Materials and Methods:** Fifty Sudanese patients with thyroid palpable lumps were investigated, without prior diagnosis. FNA was obtained and the smears were subsequently demonstrated using Papanicolaou (Pap) and Feulgen procedures. **Results:** Pap stained smears have showed 4(12%) cases with atypical malignant cells, 12 (24%) with thyroid hyperplastic cells and the remaining 34(68%) were found with normal thyroid cells.

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*Different quantifications were seen in Feulgen stained smears. **Conclusions:** Pap and Feulgen procedures are useful procedures to differentiate the different peri-neoplastic cellular pattern in thyroid lumps.*

Key words: Papanicolaou; fine needle aspiration; thyroid, Feulgen, Sudanese.

INTRODUCTION

The most common thyroid disorders, with a growing recognition globally, are the thyroid nodules and thyroiditis, which leads to an escalation of thyroid cancer incidence [1].

Amongst the frequent causes of the thyroid nodules and their transformation into thyroid cancer is the ionizing radiations, which are the most important factors that define the metaplasia of the thyroid tissue [2]. Another important cause is the environmental factors, such as the iodine deficiency in food and water, or the radiation in the soil, leading to the presence of the endemic goiter, responsible of thyroid stimulating hormone (TSH) extreme stimulation of the thyroid growth [3].

Nodular thyroid disorders designated by the existence of single or multiple nodules within the thyroid gland, remain a common clinical difficult. However, the mainstream of the thyroid nodules are non-neoplastic. To avoid needless surgery, a thyroid scan, ultrasonography, and fine needle aspiration cytology (FNAC) are used as diagnostic tools to distinguish malignant nodules from a benign lesion [4].

FNAC is an accepted technique for the basic, preoperative cytological diagnosis of thyroid nodules [5].

The routine use of FNAC in the assessment of thyroid nodules has reduced the number of patients entitled to thyroidectomy for benign disorders of the thyroid [6]. The

sensitivity, specificity, and accuracy of FNAC for malignancy detection have eclipsed the diagnostic utility of other diagnostic methods [7,8]. Various attempts have been made to improve the diagnostic accuracy of FNAC, including morphometric studies, DNA measurement, immunohistochemistry, and enzyme techniques for thyroid cancer with varying degrees of success [9]. Therefore, the aim of this study was to assess the cellular pattern of thyroid lumps in Papanicolaou and Feulgen (For DNA) stained Fine Needle Aspirates.

MATERIALS AND METHODS

This is a prospective study investigated fifty Sudanese patients presenting with thyroid palpable lumps and referred to the department of pathology at Radiation and Isotope center Khartoum (RICK), Sudan for FNA diagnosis. Efficacy of Pap staining and Feulgen were studied in FNA of the thyroid lesions along with the cytomorphological features, and results were internally controlled by materials obtained from patients with thyroid carcinoma.

The thyroid lumps were aspirated with a 23-gauge needle attached to a 20 mL disposable syringe fixed to a syringe holder. The smears were wet fixed using 95% ethyl alcohol and stained with Pap technique. Cytomorphological features including cellularity, and other architectural patterns, were assessed. Other smears were stained using Feulgen technique to find out quantitative measures of Deoxyribonucleic Acid (DNA) in different lesions.

Ten smears taken from patients with thyroid carcinoma were included to serve as internal control.

Staining: The original formula of Papanicolaou stain in which Harris Hematoxylin was used as a nuclear stain. The cytoplasmic stains were EA 50 which was essential for staining

cytoplasmic components due the action of light green and eosin yellowish stains and both were prepared in alcohol solution. The other component of cytoplasmic stains is Bismarck brown, which adds more color for differentiation. Another separate stain OG6 was used for keratin staining. The time allowed for staining was approx. 15 minutes. Difficulty in identifying cellular component from the thyroid due to over staining, was overcome by using progressive stain for 20 secant. The time allowed for the cytoplasmic staining was 4 minutes for EA 50 and 1 min for OG6.

Feulgen reaction is the standard technique for demonstrating Deoxyribonucleic Acid. Using mild acid hydrolysis by employing 1molar hydrochloric acid at 60C°, was used to break the purine –deoxyribose bond; the resulting exposed aldehydes were then demonstrated by the use of Schiff's reagent. Elements containing DNA were stained a red – purple color. The hydrolysis was the critical part of the method. An important consideration in selecting the correct hydrolysis time was the fixative used.

Ethical consent

Each participant was asked to sign a written ethical consent during the interview before the specimen was taken.

RESULTS

In the present study, FNA materials, were obtained from 50 Sudanese patients with thyroid palpable lumps, their ages ranging from 19 to 85 years with a mean age of 40 years old. The males' females' ratio was 1.00:11.50.

As shown in Table1, 60% of the study subjects were younger than 40 years; 14% were above the age of 50 years and 24% were 30 years old or less.

The FNA diagnosis of the 50 thyroid lumps were as follows: 4/50 (8%) were smears showing atypical cells as shown in microphotograph 5; 12/50(24%) were showing thyroid hyperplastic cells as shown in Photomicrograph 3, and the remaining 34/50(68%) were found with normal thyroid cells shown in Photomicrograph 1. As the number of the male was very small, most of the changes were detected among females, as shown in Table.2 and Fig.2.

Table 3. Shows the distribution of the study subjects by residence. The great majority of the study subjects were from Khartoum constituting 80% followed by North and Central States constituting 4% and 3% respectively.

Table 4. shows the distribution of the study subjects by tribes. The majority of patients were from the tribes who were located at the northern State, followed by central states and western State, constituting 42%, 30% and 22% correspondingly.

The measurement of the Intensity of Feulgen reaction for DNA can be seen in Table 5, Fig.3 and Photomicrograph 4. Ten of the smears that were taken from the patients with thyroid hyperplasia and one smear from a patient with atypical thyroid cells, have shown moderate intensity DNA staining (++) by Feulgen staining method. Notably, all smears that were diagnosed as containing normal thyroid cells (68%) and 0% of those showing atypical thyroid cells were showed with mild staining intensity(+), shown in Photomicrograph 2. Furthermore,6(60%)of the ten cases of thyroid carcinomas which were included as internal control, showed highly intense DNA staining (+++) (Photomicrograph6) and the remaining 4 (40%) have shown less staining intensity(++).

Table (1) : Distribution of the study subjects according to age

Age group	Frequency	Percent	Cumulative percent
<30	12	24.0	24.0
31-40	18	36.0	60.0
41-50	13	26.0	86.0
50+	7	14.0	100
Total	50	100	

Table (2): Distribution of the study subjects according to diagnosis and gender

Cellular pattern	Sex		Total
	Males	Females	
Normal cells	2	32	34
Hyper plastic cells	1	11	12
Atypical cells	1	3	4
Total	4	46	50

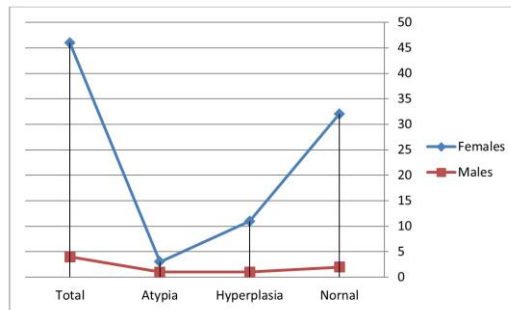


Figure (1): Description of the study subjects according to diagnosis and Sex

Table (3): Distribution of the study subjects according to diagnosis and to residence

Cellular pattern	Residence						Total
	Khartoum State	Centre ¹ State	North State	South State	East State	West State	
Normal cells	26	3	2	1	1	1	34
Hyperplastic cells	10	0	2	0	0	0	12
Atypical cells	2	0	2	0	0	0	4
Total	40	3	4	1	1	1	50

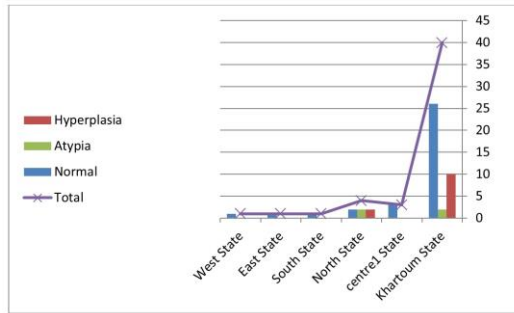


Figure 2 Description of the study subjects according to cellular pattern and residence

Table (4): Distribution of the study subjects according to diagnosis tribes

Cellular pattern	Race					Total
	middle tribes1	north tribes2	south tribes3	east tribes4	west tribes5	
Normal	10	14	2	1	7	34
Hyperplastic	4	5	0	0	3	12
Atypical	0	2	0	0	2	4
Total	15	21	2	1	11	50

1= Gamoeia, Kawahla, 2=Galia, Shwagia, Robatab, Danagla, Halfaween
 3=Denka
 4=Bani ameer
 5=Batra, Tunger, Noba, Fur, Zagawa

Table (5): Intensity of Feulgen reaction for DNA in the different degree categories

Cellular pattern	Intensity of Feulgen reaction for DNA				Total
	-	+	++	+++	
Normal cells	0	34	0	0	34
Hyperplasia	0	1	10	1	12
Atypical neoplasia	0	1	1	2	4
Thyroid carcinoma	0	0	4	6	10
Total	4	36	14	6	60

(-): no stain, (+): mild staining intensity, (++): moderate staining intensity, (+++): highly staining intensity

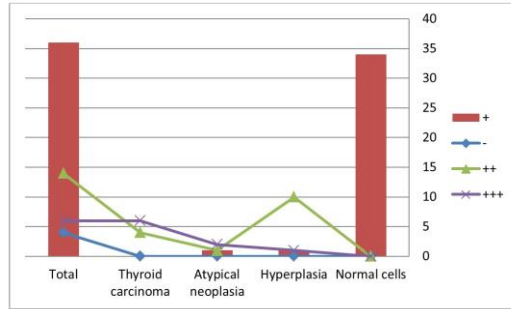
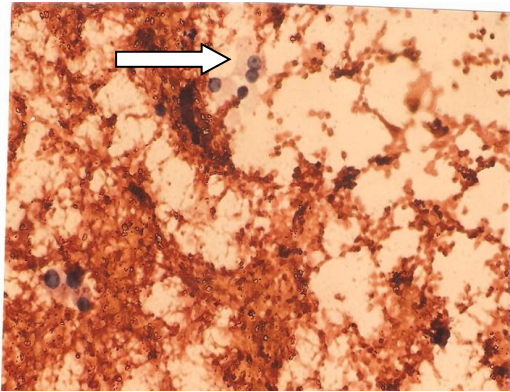
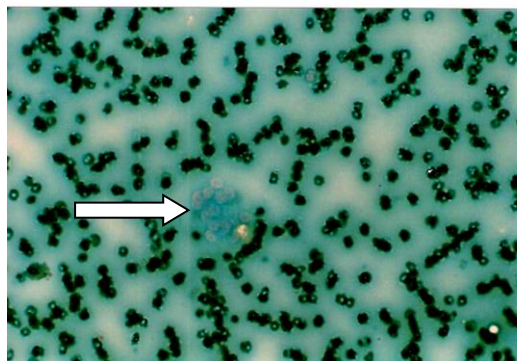


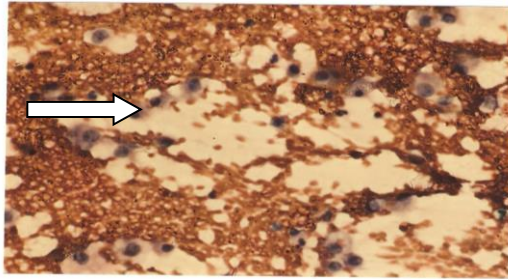
Figure (3): Description of the study subjects according to cellular pattern and to Intensity of Feulgen reaction for DNA



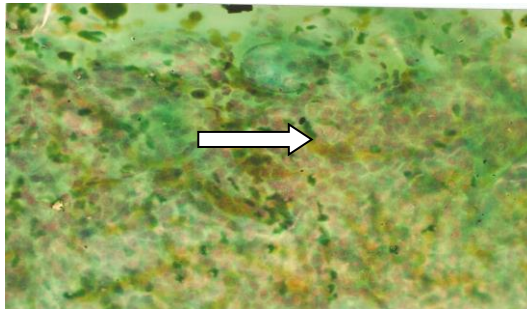
Microphotograph (1): Normal thyroid cells. Pap. Stain(x40).



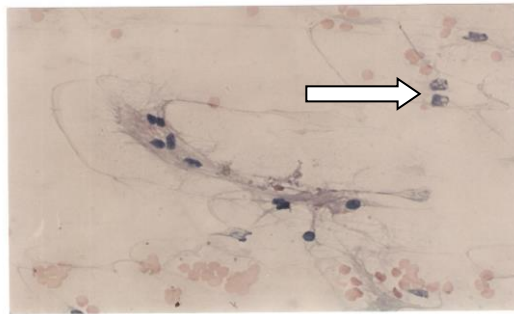
Microphotograph (2): Group of normal thyroid cells stained light magenta colour(+) by Feulgen reaction(x 40).



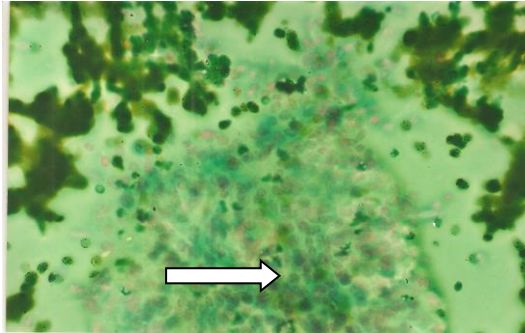
Microphotograph (3): Group of hyperplastic thyroid cells. Pap. stain (x 40).



Microphotograph (4): Group of hyperplastic thyroid cells stained moderate magenta colour (++) by Feulgen reaction (x 40).



Microphotograph (5): Atypical thyroid cells. Pap. Stain. (x 40).



Microphotograph (6): Group of neoplastic thyroid cells stained deep magenta color (+++) by Feulgen reaction. (x 40).

DISCUSSION

Globally, the prevalence of thyroid nodular lumps is very high, particularly in endemic areas[10-12]]. About 93% of all thyroid nodules are benign and the remaining 7% are thyroid carcinomas with most common papillary thyroid carcinoma [5]. The present study tried to investigate a series of Sudanese patients with thyroid nodules applying to different methods to FNA; Pap test to categorize the morphological cellular pattern and Feulgen method to quantify the DNA contents in each cellular pattern.

In this study, the incidence of atypical cells, which were categorized as per-neoplastic was found to be 8%, which was relatively similar to the global rate of the endemic areas [13]. The known prevalence rates of thyroid cancer in the African are as follows (papillary: 6.7–72.1%, follicular: 4.9–68%, anaplastic: 5–21.4%, and medullary: 2.6%–13.8%) [14]. In study from western Sudan where goiter is endemic, the prevalence of thyroid cancer was found to be 5.7% among 246 patients [15].

In the current study 24% of the FNAs have shown metaplasia which may indicate the benign neoplastic desire. This along with presence of a reasonable number of normal cellular indicates the diversity of these lesions among Sudanese

patients. Among 246 patients with thyroid nodules, 60.2% of the operations were for endemic multi-nodular goiter mainly for pressure symptoms or signs, 14.6% for solitary thyroid nodules, 13% for simple diffused goiter, 6.5% for toxic goiter [15]. As in the literature the gathering many studies from different geographical regions, the great majority of the patients are females.

The value of FNAC for diagnosis of thyroid lumps has been established; however, in some specific situations, its reliability is controversial. Although, this diagnostic method is simple, cost-effective and valuable for the assessment of single thyroid lesions, but has shown less efficacy for that of multi-nodular thyroid glands. Therefore, in this study additional method was used to quantify the qualitative assessment of conventional Pap cytomorphological pattern. Quantification of DNA using Feulgen reaction is useful to indicate cellular proliferative activity which was apparent in the present study in form of staining intensity. This attempt may be of a value to stimulate future directions in this context which may be simple, reliable and cost-effective.

The strength in the present study was the use of the internal control from patients who were previously confirmed as having thyroid cancer, but the limitation is the absence of histopathology as a gold standard.

CONCLUSION:

Thyroid proliferative changes are common among Sudanese patients with thyroid nodular disorders. DNA assessment using Feulgen reaction is promising diagnostic tool to change FNA evaluation from qualitative which depends on individual experience and skill to quantitative measure.

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