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Original Research

Application of Buccal smear for detection of Cytological changes Of Hormonal Therapy

Fadwa Mohammed Ebraheem[1], Alkhair Abd Almahmoud Idris[2,*]

ABSTRACT

Background: Cancer-related mortality is high in Sudan, with low survival rates attributed to advanced-stage disease at presentation. This study aimed to assess hormonal therapy-induced cytological changes in buccal mucosa among Sudanese women with breast and endometrial cancers. **Methods**: This community-based, quantitative study evaluated cytological changes in buccal smears from women undergoing hormone therapy in Khartoum State. Fifty patients receiving hormone therapy were included. Buccal smears were collected from participants and prepared using wet-fixation and air-dried methods for staining with hematoxylin and eosin (H&E). **Results**: Cytological analysis revealed malignant changes in 56% of cases, inflammatory changes in 34%, and normal findings in 10%. Results were comparable between wet-fixation and air-dried methods. Smear quality for the wet method was rated as excellent (40%), very good (28%), good (20%), acceptable (8%), and poor (4%); similar quality distributions were observed for the dry method. No significant differences in cytological findings (P = 0.14) or smear quality (P = 0.11) were observed between preparation methods. However, cytological abnormalities significantly correlated with duration and frequency of hormone therapy (P = 0.001).**Conclusion:** Buccal smears from women undergoing hormone therapy predominantly exhibited malignant cytological changes, followed by inflammatory and normal findings. Preparation method (wet vs. dry) did not significantly influence outcomes.

Key words: Cytological results, Buccal smears, Sudan, hormone

INTRODUCTION

Chemotherapy and radiation are the most widely used interventions for the treatment of cancer [1–4]. Oral complications that arise with chemotherapy and/or radiation therapy induce many disorders represented in many scientific reports [2,5–10]. Hormone replacement therapy (HRT) has long been recognized as a method of treatment for adverse symptoms resulting from the cessation of ovarian endocrine function [10]. Most patients in Sudan receive high and repeated doses of hormonal replacement therapy. This study was designed to assess the changes in buccal smears induced by hormonal replacement therapy among Sudanese women with breast and endometrial cancers. The study aimed to compare between wet fixed and air-dry Hematoxylin and Eosin (H&E) stained buccal smear samples in diagnosing buccal cytology changes for the detection of buccal cytology changes due to hormonal therapy among Sudanese women with breast and endometrial cancers.

METHODS

This was a community-based, quantitative study to assess the cytological changes in buccal cavity smears among patients undergoing hormone replacement therapy in Khartoum State. The study included 50 patients under hormone therapy and was carried out in Khartoum State, Sudan, among women who underwent hormone therapy.

Study Sampling

Buccal smear samples were collected from one hundred participants to prepare for staining with H&E stain using wet and dry methods.

Sample Collection

Collect one buccal smear from the buccal mucosa of patients undergoing hormone replacement therapy using a tongue depressor after washing the mouth to avoid contamination by bacteria.

Sample Procedure

For wet fixed smears, the smear was taken using the brush method and stained in Mayer's hemalum solution for 3 minutes, then rinsed in 0.1% HCl solution

[1]*Gharb El-Niel College-Sudan*

[2]*Ahfad University for Women, Sudan*

Correspondence

Alkhair Abd Almahmoud Idris, Ahfad University for Women, Sudan

Email: alkhair20@hotmail.com

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for 2 seconds. The slide was differentiated in running tap water for 3-5 minutes, then stained in 0.5% aqueous solution of eosin Y for 3-5 minutes, rinsed in tap water for 3 seconds, in an ascending alcohol series, Xylene was applied, then the smear was mounted in DPX.

Dry Smears

Slides were allowed to air dry for several minutes to remove moisture, then stained with filtered 0.1% Mayer's Haematoxylin for 10 minutes in a 50ml conical tube in a Coplin jar, slides were rinsed in cool running water for 5 minutes. The slides were then stained in 0.5% eosin (1.5g dissolved in 300ml of 95% ethanol) for 12 times, dipped in distilled water until the eosin stopped streaking, applied in 50% ethanol for 10 minutes and in 70% ethanol for 10 minutes, equilibrated in 95% ethanol for 30 seconds, and equilibrated in 100% ethanol for 1 minute. Finally, the slides were dipped in Xylene several times, mounted, and cover slipped for examination.

Statistical Analysis

After examining the smears under a microscope, the results of the laboratory investigation as well as demographic data from the patients will be processed using the Statistical Package for Social Science (SPSS).

Ethical Approval and Consent to Participate

Ethical approval was obtained from the Gharb El-Niel College Ethical Research Committee in accordance with the Declaration of Helsinki Principles, and agreements were obtained from hospital administrations before sample and data collection. Patient information was highly secured and not used for purposes other than scientific inquiry.

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RESULTS

Table 1 shows that 42% of the patients in the age group 36-45 years and 12% in the age group 20-25 years.

Table 2 shows that 32% of the patients have secondary level ofeducation and 18% were illiterates.

Duration of the hormonal therapy received by the patients is shown in **Table 3**. Majority 40% use hormonal therapy for more than 9 years, 34% 3-9 years, 26% less than 3 years.

Frequency of the hormonal therapy dose per day that received by thepatients is shown in **Table 5**. Majority (52%) used it 3 times per day, 38% twice per day, and 20% once per day.

As shown in **Table 5** the cytological assessment of buccal smears prepared by we fixed tmethod were malignant (56%), inflammatory (34%) and normal (10%) and the same results obtained by dry method. No significant statistical differences in cytopathological assessment of the buccal smears prepared by dry and wet methods (P = 0.14, > 0.05).

As shown in **Table 6** the slide staining quality prepared by wet fixed method were excellent (40%), very good (28%), good (20%), acceptable (8%) and poor (4%), and the same results obtained by dry method. No significant statistical differences in the slide staining quality prepared by dry and wet fixed methods (P 0.11,> 0.05).

As shown in **Table 7** the malignant cells of buccal smears reported in 7.7%, 47.1% and 95% among patients used hormonal therapy for <3 years, 3-9 years and > 9 years respectively. Significant differences were found in pathological assessment of the buccal smears in relation to duration of use of hormonal therapy (P =0.00 1, <0.05).

As shown in **Table 8** the malignant cells of buccal smears reported in 20%, 21.1% and 88.5% among patients used hormonal therapy within frequency of 1 per day, 2 per day and 3 times per day respectively. Significant statistical differences were found in pathological assessment of the buccal smears in relation to frequency of use of hormonal therapy per day (P 0.002, <0.05).

DISCUSSION

In this study, fifty Sudanese women who received hormone therapy were assessed based on cytological changes in buccal smears using wet fixed and dry H&E stains. A previous study suggested that patients exposed to cancer therapy exhibit poorer oral health[11]. As shown in **Table 5**, the pathology of buccal smears prepared by the wet fixed method were malignant (56%), inflammatory (34%), and normal (10%), with similar results obtained by the dry method. There were no significant differences in the pathology of the buccal smears prepared by dry and wet methods (P=0.14>0.05). **Table 6** displays the slide staining quality of buccal smears prepared by the wet method as excellent (40%), very good (28%), good (20%), acceptable (8%), and poor (4%), with the same results obtained by the dry method. No significant difference was found in the slide staining quality of the buccal smears prepared by dry and wet

Table 1: Distribution of the patients according to age

Age	Number	Percentage %
20-25 years	6	12.0
26-35 years	14	28.0
36-45 years	21	42.0
45-50 years	9	18.0
Total	50	100.0

Table 2: Distribution of the patients according to educational level

Educational background	Number	Percentage %
Illiterate	9	18.0
Primary	11	22.0
Secondary	16	32.0
University	14	28.0
Total	50	100.0

Table 3: Distribution of the patients according to duration of hormonal therapy

Duration of hormone therapy	Number	Percentage %
<3 years	13	26.0
3-9 years	17	34.0
>9 years	20	40.0
Total	50	100.0

Table 4: Distribution of the patients according to frequency of Hormonal therapy dose

Frequency of hormonal therapy dose	Number	Percentage %
Once per day	5	10.0
Two times a day	19	38.0
3 times a day	26	52.0
Total	50	100.0

Table 5: Distribution of the patients according to cytology results

Pathology	Wet fixed smear		Dry smear		P value
	Number	Percentage %	Number	Percentage %	
Normal	5	10.0	5	10.0	0.14
Inflammatory	17	34.0	17	34.0	
Malignant	28	56.0	28	56.0	
Total	50	100.0	50	100.0	

Table 6: Distribution of the cytological results according to slide staining quality

Slide staining quality	Wet fixed smear		Dry smear		P value
	Number	Percentage %	Number	Percentage %	
Excellent	20	40.0	20	40.0	0.11
Very good	14	28.0	14	28.0	
Good	10	20.0	10	20.0	
Acceptable	4	8.0	4	8.0	
Poor	2	4.0	2	4.0	
Total	50	100.0	50	100.0	

Table 7: Correlation between cytology results and duration of hormonal therapy use

Cytology results	Duration						P value
	>3 years		3-9 years		>9 years		
	Number	Percentage %	Number	Percentage %	Number	Percentage %	
Normal	4	30.8	0	0.0	1	5.0	0.001
Inflammatory	8	61.5	9	52.9	0	0.0	
Malignant	1	7.7	8	47.1	19	95.0	
Total	50	100.0	50	100.0	50	100.0	

Table 8: Correlation between cytological results in relation to dose of hormonal therapy used per day

Cytology results	Duration						P value
	1 \ day		2 \ day		3 \ day		
	Number	Percentage %	Number	Percentage %	Number	Percentage %	
Normal	2	40.0	2	10.5	1	3.8	0.002
Inflammatory	2	40.0	13	68.4	2	7.7	
Malignant	1	20.0	4	21.1	23	88.5	
Total	50	100.0	50	100.0	50	100.0	

methods (P=0.11>0.05). This is in agreement with some previous reports [4,10,12].

Table 7 shows the malignant pathology of buccal smears prepared by wet and dry methods reported in 7.7%, 47%, and 95% of the smears obtained from patients who used hormonal therapy for <3 years, 3-9 years, and >9 years, respectively. A significant difference was found in the pathology of the buccal smears according to the duration of use.

In **Table 8**, the malignant cells of buccal smears prepared by wet fixation and dry methods were reported in 20%, 21.1%, and 88.5% of the smears obtained from patients who used hormonal therapy 1, 2, and 3 times per day, respectively. Significant differences were found in the pathology of the buccal smears according to the frequency of use per day of hormonal therapy (P = 0.002 < 0.05). These results are in agreement with previous findings [7,13]. A small sample size and the limitation of only sampling from Khartoum State in Sudan, without including other cities, are the present limitations.

CONCLUSION

The study concluded that the cytological results of buccal smears prepared by wet fixation and dry methods were more likely to be malignant, followed by inflammatory and normal findings. There were no significant differences in the cytological reports of the buccal smears prepared by dry and wet methods. Malignant buccal cells showed significant differences in the buccal smears according to the duration and frequency of hormone therapy use per day.

ABBREVIATIONS

HRT: Hormone replacement therapy
H&E: Hematoxylin and Eosin
SPSS: Statistical Package for Social Science

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

Authors declare that they have no competing interests.

FUNDING

Not applicable.

AUTHORS' CONTRIBUTIONS

FME and AAI conceived the design and carried out the experiments. FME obtained, analyzed and interpreted the data. FME and AAI wrote and revised the manuscript. AAI provides financial support for all experiments. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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