



Screening Buccal Smear to Identify Cytological Alterations Brought on by Hormone Therapy

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Abstract Objective: To assess the differences in buccal cytology changes caused by hormone therapy in Sudanese women with breast and endometrial malignancies by comparing wet fixed and air dry H&E stained buccal smear samples. Method: The eight-month study period ran from October 2015 to May 2016. The practical inquiry began in November 2015, and during that time, data gathering, a literature review, and research script typing were completed. One hundred participants had buccal smear samples taken, and these were ready for H&E staining using a wet and dry procedure on women receiving hormone medication. Results: The wet method produced buccal smears with pathology that was 56% malignant, 34% inflammatory, and 10% normal; the dry method produced identical results. The wet method produced excellent (40%), very well (28%), well (20%), acceptable (8%) and bad (4%) affinity, which was in line with the results of the dry method. There were no appreciable variations in the pathology and affinity of the wet and dry-prepared buccal smears ($P = 0.14$ and $0.0011 > 0.05$). The cancerous The pathology of wet and dry buccal smears was reported in 7.7%, 47.1%, and 95% of the smears obtained from patients who had been on hormonal therapy for less than three years, three to nine years, and more than nine years, respectively. Wet and dry smears were reported in 20%, 21.1%, and 88.5% of the smears obtained from patients who had been on hormonal therapy once, twice, or three times a day, respectively. Conclusion: The pathology of the buccal smears showed significant differences based on the frequency and duration of hormone therapy.

Keyword : Buccal Smear Screening, Cytology Changes, Hormone Therapy

1. INTRODUCTION

The maxillary and mandibular vestibular folds form the buccal mucosa's vertical borders, while the anterior pillar and outer commissural of the lips form its anterior and posterior borders, respectively. The two most commonly employed interventions for the treatment of cancer are chemotherapy and radiation therapy. Despite being used to enhance the patient's quality of life, these treatments come with a number of drawbacks. Numerous unfavorable responses stem from these treatments. Furthermore, they also have an impact on the patient's financial situation. Approximately 400,000 occurrences of oral cavity injury caused by treatments occur each year.

Oral complications that arise with chemotherapy and /or radiation therapy induce mucositis (stomatitis); xerostomia (dry mouth); dental caries; loss of taste; bacterial, fungal, or viral infection (especially in patients who are neutropenic). A serious non-hematologic side effect of cytotoxic chemotherapy and radiation therapy is oral mucositis, which is linked to severe morbidity such as discomfort, odynodysphagia, dyseugia, and the ensuing dehydration and malnourishment.

A number of oral toxicities may also impair the effectiveness of recommended cancer treatment regimens. To enable the clearance of oral lesions, for instance, dosage reductions or adjustments to the treatment plan can be required. When a patient develops many oral morbidities, they may become incapable of receiving cancer treatments; in such circumstances, treatment is typically stopped. Oral problems can cause dose interruptions that have a direct impact on patient survival.

Hormone replacement therapy, or HRT, has long been acknowledged as a therapeutic option for treating unfavorable symptoms brought on by the suppression of ovarian endocrine activity. Hormone replacement treatment (HRT) can be given as estrogen-only or estrogen-progesterone therapy. Research over the past few years has shown that hormone replacement therapy (HRT) can help prevent distant menopausal consequences including osteoporosis and skin atrophy. Postmenopausal bone loss can be significantly or totally avoided with estrogen replacement therapy.

Sudan has a high cancer death rate and low cancer survival rates since the majority of patients arrive with advanced disease. Hormone replacement treatment is administered in large dosages to the majority of patients. Exfoliative cytology is commonly utilized for oral mucosal lesion screening and diagnosis; it can also be used, and is favored above, clinical assessment for tracking changes due to therapy. The purpose of this study was to evaluate the effects of hormone replacement treatment on the buccal smears of Sudanese women with breast endometrial cancer.

2. LITERATURE REVIEW

Oral mucosa:

Known by many other names, the oral cavity, or mouth, is a complex anatomical structure made up of both soft and hard tissue components. It can be found close to where the gastrointestinal tract begins. It might be argued that a person's oral health is a good indicator of their general health. The local effects of long-term alcohol or tobacco use, as well as illnesses like diabetes or vitamin deficiencies, are thought to be the causes of changes in the oral mucosa. Protecting the underlying structures from harm, as well as against the admission of certain microbes and potentially harmful substances into the oral cavity, is the primary function of the oral mucosa. The stratified squamous epithelium covers the soft tissue of the human mouth cavity and esophagus. The oral mucosa is closely linked to the underlying collagenous connective tissue,

known as lamina propria. Mucosal areas in the oral cavity differ in terms of thickness, origin, and epithelial maturation stage.

A keratinizing epithelium covers the mucosa in areas subjected to mechanical pressures and linked with mastication (e.g., gingiva and hard palate). In some areas of the oral cavity, aging produces modest epithelium thinning, as well as flattening of the epithelial-connective tissue junction. In female patients, aging has also been demonstrated to reduce the permeability of the mucosa on the floor of the mouth. The oral buccal epithelia are microscopically identical. Surface keratinization patterns, as well as the distribution and appearance of lipid lamellae in intercellular gaps, were shown to be similar in vaginal and buccal epithelial samples from postmenopausal women. The two epithelia had identical lipid compositions, with the exception of cholesterol esters and glycosylceramides, which were more plentiful in the buccal epithelium. The response of oral epithelium to hormones and HT is unclear Croley and Miers (1978) discovered that patients with high estrogen levels had more superficial and keratinized epithelial cells in their buccal smears, whereas those with higher progesterone levels had more intermediate epithelial cells and fewer keratinized cells. As a result, alterations similar to those shown in research on vaginal microbes may occur in the mouth microbiome. However, oral lactobacilli strains differ from those found in vaginal microbiome. In comparison to premenopausal women, postmenopausal women have a lower concentration of vaginal lactobacilli and a higher concentration of vaginal E.coli.

Endometrial cancer:

Endometrial cancer is the most well-documented consequence of HTR in women who have not had a hysterectomy. The lifetime incidence of endometrial cancer in the general population is about 3%. Endometrial cancer risk is estimated to be 4 to 10 times higher in estrogen-only users with an intact uterus compared to non-users. However, no equivalent rise in mortality from endometrial cancer has been documented, possibly due to improved surveillance and identification. Women who take estrogen replacement medication are also more likely to have well-differentiated and less invasive malignancies. those who have undergone a hysterectomy can be treated with estrogen alone; however, those who have an intact uterus must be treated with estrogen and progestogen HRT due to an elevated relative risk of endometrial cancer. This risk is endometrial cancer. This risk is significantly lowered by the addition of a progestogen, either cyclically or continuously.

Some studies found that estrogen plus progestogen HTR significantly reduced the risk of endometrial cancer compared to estrogen replacement treatment alone. However, one study found a relative risk greater than 3 (confidence interval, 1.7 to 5.7) among women who had been taking HTR for more than 5 years and had less than 10 days of progestogen each month.

Breast Cancer:

Fear of breast cancer is one of the leading reasons patients cease taking HTR, and many physicians are hesitant to prescribe HTR; however, the information on breast cancer is inconsistent. Although it is increasingly obvious that HTR does not reduce the risk of breast cancer, there is still no agreement on whether or how much HTR raises the risk of breast cancer.[18]In women who had been using estrogen replacement for five years or more, the relative risk increased marginally to 1.46 (confidence interval, 1.22 to 1.74). The inclusion of progestin did not materially alter the reported relative risks.

Personal History:

A personal history of breast cancer is an unequivocal contraindication for HTR. Only a few research have looked into the role of HTR in women with a personal history of breast cancer because to the presumed theoretical danger. One study tracked 49 women with a history of breast cancer on HTR for an average of 31 months.

Family History:

A family history of breast cancer is not a contraindication for HTR. Although patients with a family history of breast cancer may be hesitant to use HTR, this medication is unlikely to significantly raise their risk of breast cancer.

Hormone Replacement Therapy (HTR):

Hormone replacement therapy (HTR) is any form of hormone therapy wherein the patient, in the course of medical treatment, receives hormones, either to supplement a lack of naturally occurring hormones, or to substitute other hormones for naturally occurring hormones. Common forms of hormone replacement therapy include. : Hormone replacement therapy for menopause is based on the idea that the treatment may prevent discomfort caused by diminished circulating estrogen and progesterone hormones, or in the case of the surgically or prematurely menopausal, that it may prolong life and may reduce incidence of dementia. It involves the use of one or more of a group of medications designed to artificially boost hormone levels. The main type of hormone involved is estrogen and progesterone or progestin, and sometimes testosterone it is often referred

to as "treatment" rather than therapy. Hormone replacement therapy for transgender people introduces hormone associated with the gender that the patient identifies with (notably testosterone for Trans men and estrogen for Trans women). Some intersex people may also receive HTR. Cross – sex hormone treatment for transgender individuals is divided in to two main types: hormone replacement therapy (female – to male). Androgen replacement therapy (andropausal and ergogenic use) is a hormone treatment often prescribed to counter the effects of male hypogonadism. It is also prescribed to lessen the effects or delay the onset of normal male aging. Additionally, androgen replacement therapy is used for men who have lost their testicular function to disease, cancer, or other causes.

General Effects on Females:

As recently as 2005 biological women have had a positive attitude towards replacement therapy but based on the empirical data these attitudes may be overly optimistic. There is still much to learn about how HTR affects people. In the combined hormone trial, the WHI tested only estrogen (Premarin) and one progestin (Provera), in a single pill (Prempro), at a single dose (0.625 mg Provera). therefore the results are not reliable nor representative. To avoid HTR risks it is essential to use the most effective delivery method of both estrogen and progesterone. Bio identical estradiol (estrogen) when taken orally is converted in the liver to estrone, a weaker Bio identical estradiol. However when estrogen as estradiol is used transdermally as patch, gel, or passerby, it enters the bloodstream as Bio identical estradiol. When estrogen is ingested it is subjected to first pass metabolism and is processed through the liver. This first pass metabolism stimulates proteins associated with heart disease and stroke, such as C- reactive protein, activated protein C, and clotting factors. Using a patch, gel or passerby to take estrogens avoids first pass metabolism and the risks associated with it and the same level of blood concentration can be achieved avoiding the serious side effects associated with oral estradiol HTR. Current research shows that transdermal route of estradiol administration can be advantageous for women with diabetes, hypertension and other cardiovascular risk factors, as those risks increase with advancing age. women taking Bio identical estrogen, orally or transdermally, who have a uterus must still take an FDA-approved progesterone or micronized progesterone to lower the risk of endometrial cancer. The natural, plant- derived progesterone creams sold over the counter contain too little progesterone to be effective. Wild yam (*Dioscorea villosa*) extract creams are not effective since the natural progesterone present in the extract is not bioavailable.

Effect of Hormone Replacement Therapy on oral cytology:

Literature was also reviewed to explore the benefit of hormone (estrogen/progesterone) replacement therapy (HTR) on oral symptoms and signs in postmenopausal women. conjugated estrogen were administered to one group of postmenopausal women with oral discomforts. They observed that HTR improved subjective and objective symptoms in more than 50% of patients. Forabosco *et al.*(2012) evaluate effect of HTR on symptoms of oral discomfort in postmenopausal women and concluded that oral discomfort may be related to steroid hormone withdrawal only in some postmenopausal women and treatment with estrogen may improve the clinical picture in this group of women only. Immunohistochemical identification of estrogen receptors may help to patients for whom HTR may be beneficial. Tarkkila *et al.* (2001) used a questionnaire to investigate the prevalence of self – assessed sensations of painful mouth and dry mouth in 3173 menopausal women. They observe that the symptoms of pain and dryness of the mouth were associated with climacteric symptoms in general but the use of HTR did not prevent or improve the symptoms. Menopause leads to decrease in salivary flow rates and alteration in levels of electrolytes in the saliva. Yalcin *et al.* (2005) did not observe any difference in salivary pH or electrolyte levels in saliva in postmenopausal women with or without HTR. Though there was no effect of alendronate and calcium supplements on these salivary parameters, hormone replacement along with alendronate and calcium improved saliva flow rate in women with oral symptoms.

3. MATERIAL AND METHODS

Buccal Smear technique:

Rinse and brush the patient's teeth to eliminate any cellular debris or foreign material from their mouth and mucosa surface. Write the patient's surname on the four frosted-end glass slides. Scrape the buccal and inner cheek surfaces vigorously with the edge of a tongue depressor. The collected material is quickly spread in this smear on the prepared slides. The specimen should be fixed immediately with spray fixative or immersed in 95% ethyl (or reagent) alcohol. Collect one buccal swab from the buccal mucosa of hormone replacement treatment patients using a tongue depressor before washing their mouth to avoid bacterial contamination.

Haematoxylin and eosin (H&E):

Haematoxylin and eosin is one of the principal stain in histology and cytology is the most widely used stain in medical diagnosis and is often the gold standard. The staining method involves

application of haem alum a complex formed from aluminum iron and hematein can oxidation product of Haematoxylin, hem alum colors nuclei of cells, the nuclear staining is followed by counter staining with an aqueous or alcoholic solution of eosin yellow, which colors eosinophilic structures in various shades of red, pink.

Sample Procedure:

Slides were fixated and stained with H&E (wet-fixed smear). For the wet research, aspirated material was expressed onto slides, and a drop of toluidine blue stain was set aside. Materials were combined with the stain, covered with a cover slip, and examined under a microscope.

Wet smears were taken with a brush, stained with Mayer's hem alum solution for 3 minutes, and washed with 0.1% HCL solution for 2 seconds. The slide was differentiated in running tap water for 3-5 minutes, then stained in 0.5% aqueous eosin Y solution for 3-5 minutes, washed in tap water for 3 seconds, ascending alcohol series, Xylene was applied, and the smear was mounted in DPX.

Dry smear:

Slides was put in Air as dry section for several minutes to remove moisture, then stained with filtered 0.1% Mayer's Haematoxylin for 10 mint in 50ml conical tube in a coplin jar, slide were rinsed in cool running of H2O for 5mint. The slide were applied in 0.5% Eosin (1,5g dissolved in 300ml of 95% Ethanol) for 12 times, after that dipped in distilled H2O until the eosin stops streaking, applied in 50% EtOH for 10 mint and in 70% EtOH for 10mint, the slide were equilibrated in 95% EtOH for 30 second; they equilibrated in 100% EtOH for 1mint. The slide finally dipped in Xylene several times, mount and cover slip to examine it.

Statistical analysis:

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

4. RESULTS

Table (1) Distribution of the patients according to age

Age	n	0.0%
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

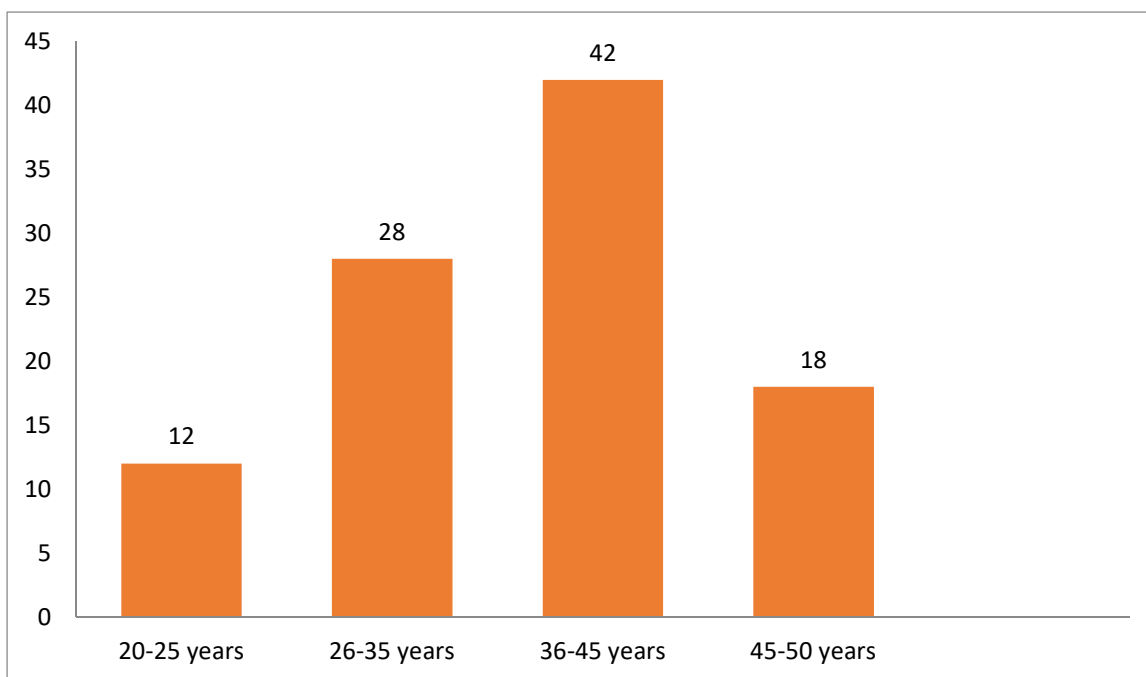


Figure (1) Distribution of the patients according to age

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) Distribution of the patients according to educational level

Age	N	0.0%
Illiterate	9	18.0
Primary	11	22.0
Secondary	16	32.0
University	14	28.0
Total	50	100.0

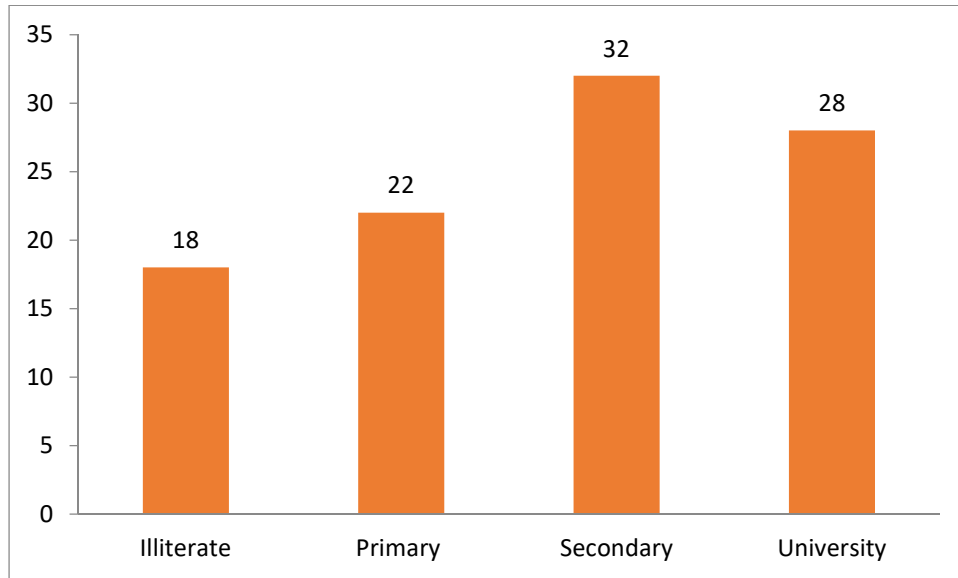


Figure (2) Distribution of the patients according to educational level

Figure (2) shows that 32% of the patients have secondary level of education and 18% were illiterates.

Table (3) Distribution of the patients according to tribal origin

Age	N	0.0%
Central	27	54.0
Eastern	7	14.0
Notliern	10	20.0
Western	6	12.0
Total	50	100.0

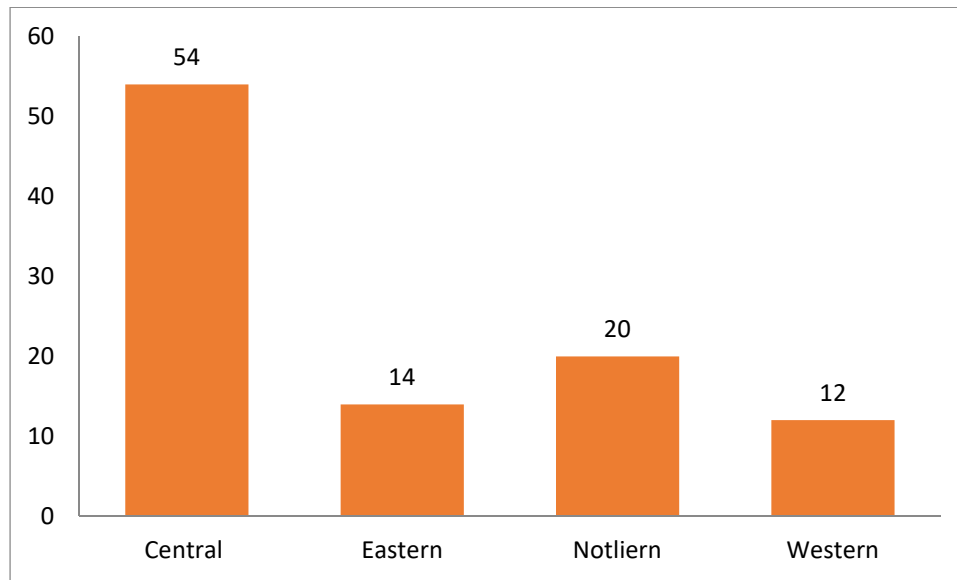


Figure (3) Distribution of the patients according to tribal origin

Tribal origin of the patients is shown in Table (3). Majority 54% from central tribes, 20% Northern tribes, 14% eastern tribes and 12% from western tribes.

Table (4) Distribution of the patients according to duration of hormonal therapy

Age	N	0.0%
<3 years	13	26.0
3-9 years	17	34.0
>9 years	20	40.0
Total	50	100.0

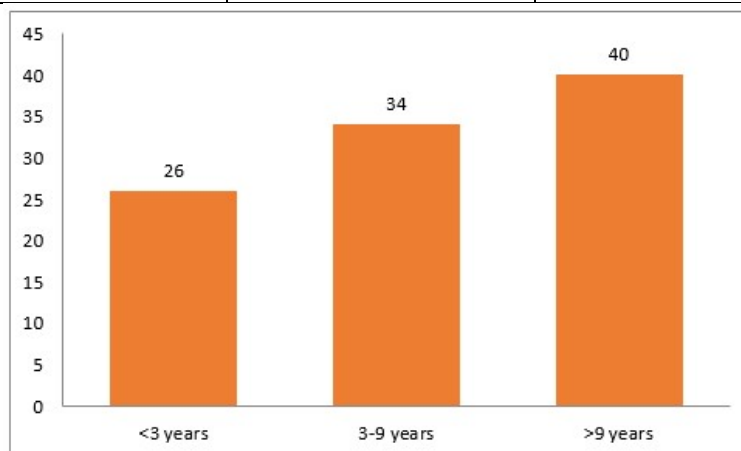


Figure (4) Distribution of the patients according to duration of hormonal therapy

Duration of the hormonal therapy received by the patients is shown in **Table (4)**. Majority 40% within more than 9 years, 34% 3-9 years, 26% less than 3 years.

Table (5) Distribution of the patients according to frequency of hormonal therapy dose

Age	N	0.0%
Once per day	5	10.0
Two times a day	19	38.0
3 times a day	26	52.0
Total	50	100.0

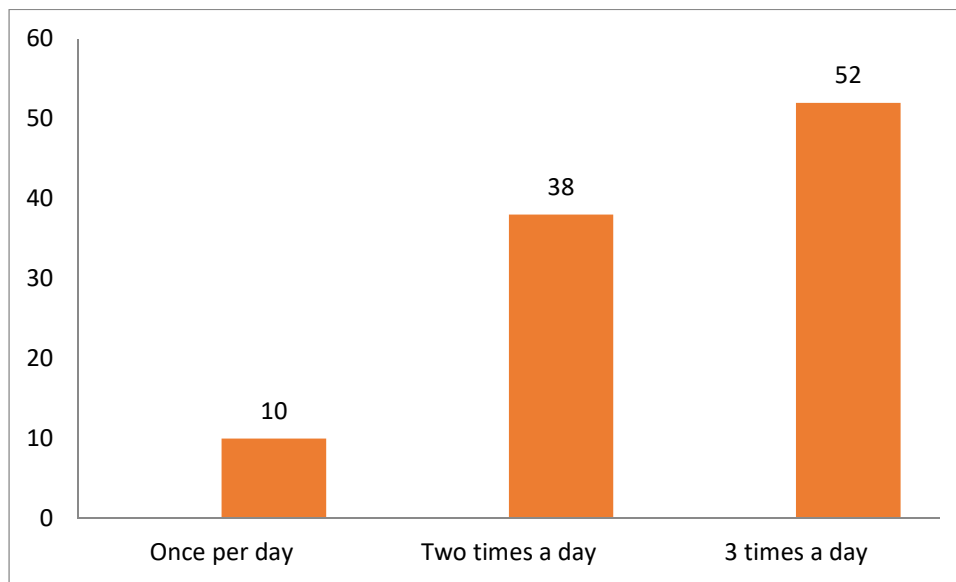


Figure (5) Distribution of the patients according to frequency of hormonal therapy dose.

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

Table (6) Distribution of the patients according to lab results (pathology of buccal smears)

Pathology	Wet		Dry		P value
	N	%	N	%	
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	

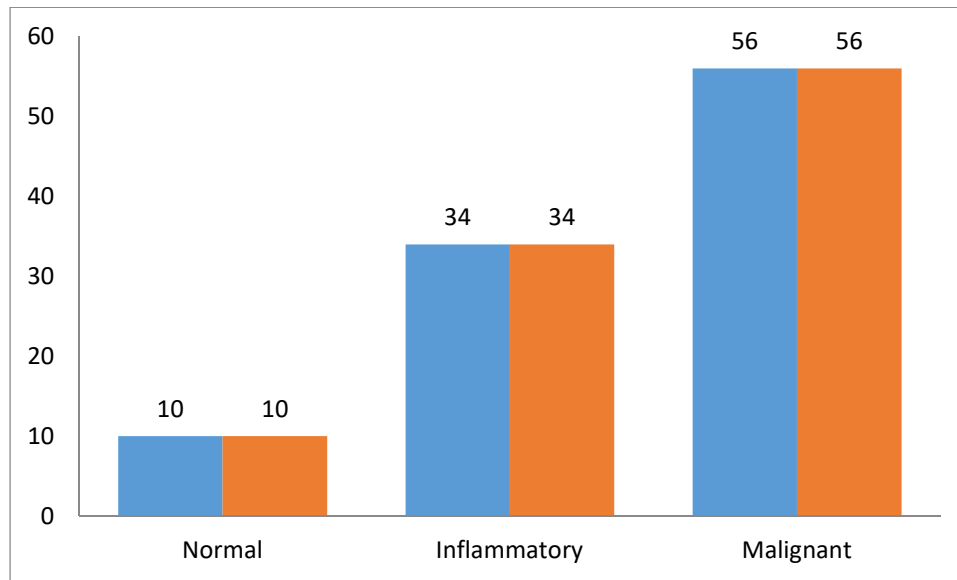


Figure (6) Distribution of the patients according to lab results (pathology of buccal smears)

As shown in Table (6) the pathology of buccal smears prepared by wet method were malignant (56%), inflammatory (34%) and normal (10%) and the same results obtained by dry method. No significant differences in pathology of the buccal smears prepared by dry and wet methods ($P = 0.14 > 0.05$).

Table (7) Distribution of the patients according to lab results (affinity of smears)

Affinity	Wet		Dry		P value
	N	%	N	%	
Excellent	20	40.0	20	40.0	0.11
Vers good	14	28.0	14	28.0	
Good	10	20.0	10	20.0	
Acceptable	4	8.0	4	8.0	
Bad	2	4.0	2	4.0	
Total	50	100.0	50	100.0	

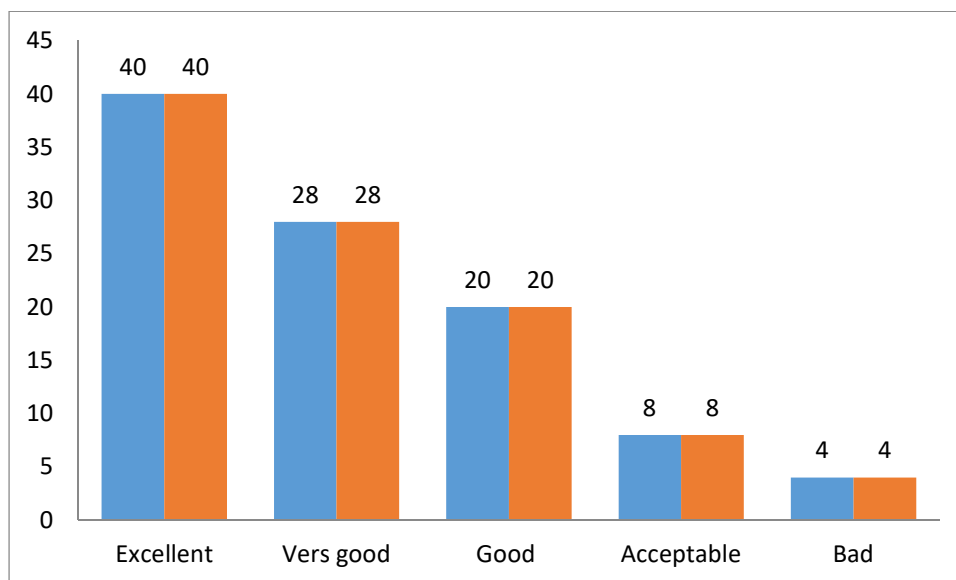


Figure (7) Distribution of the patients according to lab results (affinity of buccal smears)

As shown in Table (7) the affinity of liver cytology prepared by wet method were excellent (40%), very good (28%), good (20%), acceptable (8%) and bad (4%) and the same results obtained by dry method. No significant differences in affinity of the buccal smears prepared by dry and wet methods ($P 0.11 > 0.05$).

Table (8) Distribution of the patients according to pathology of buccal smears in relation to duration of hormonal therapy

Affinity	Duration						P value
	>3 years		3-9 years		>9 years		
	N	%	N	%	N	%	
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	

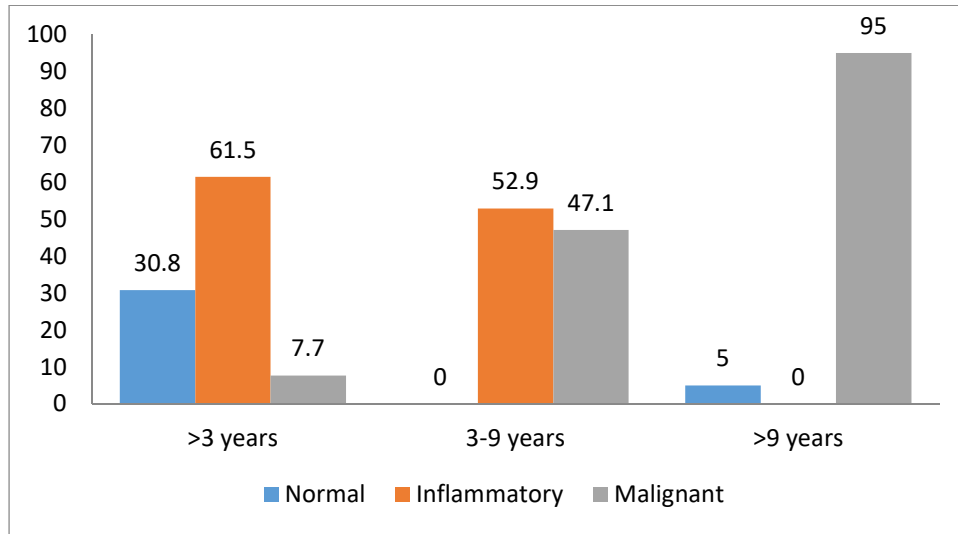


Figure (8) Distribution of the patients according to pathology of buccal smears in relation to duration of hormonal therapy

As shown in Table (8) the malignant pathology of buccal smears prepared by wet and dry reported in 7.7%, 47.1% and 95% of the smears obtained from patients used hormonal therapy for <3 years, 3-9 years and > 9 years respectively. Significant differences were found in pathology of the buccal smears according to duration of use of hormonal therapy ($P = 0.001 < 0.05$).

Table (9) Distribution of the patients according to pathology of smears in relation to frequency per day of dose of hormonal therapy

Pathology	Duration						P value
	1 \ day		2 \ day		3 \ day		
	N	%	N	%	N	%	
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	

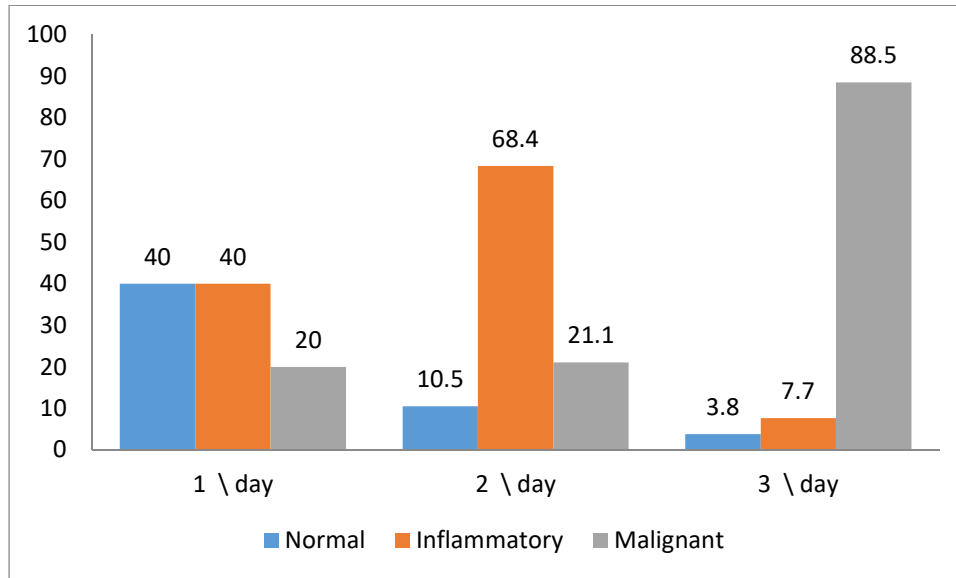
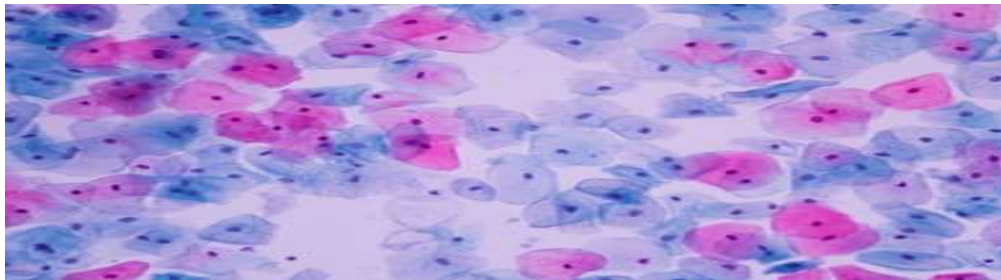
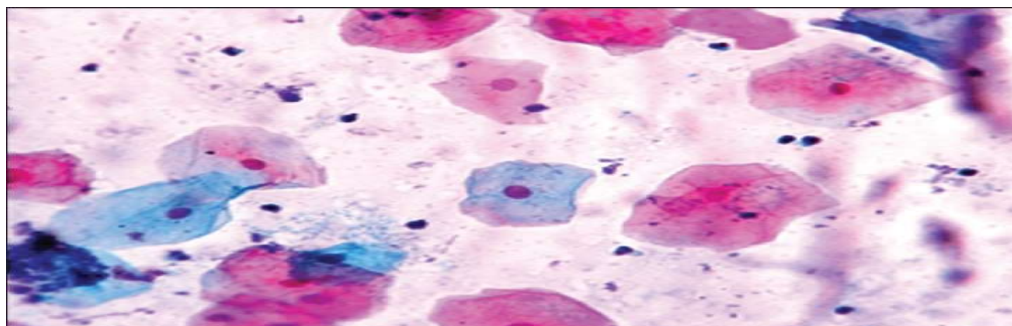


Figure (9) Distribution of the patients according to pathology of smears in relation to frequency per day of dose of hormonal therapy

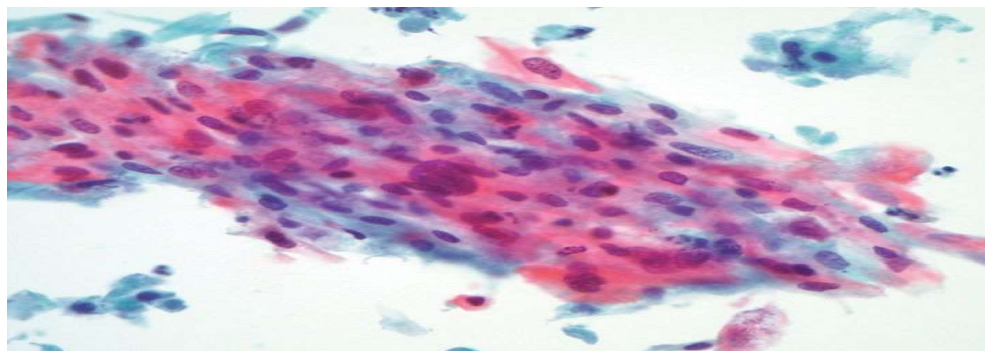
As shown in Table (9) the malignant pathology of buccal smears prepared by wet and dry reported in 20%, 21.1% and 88.5% of the smears obtained from patients used hormonal therapy in frequency of 1 per day, 2 per day and 3 times per day respectively. Significant differences were find in pathology of the buccal smears according to frequency of use per day of hormonal therapy (P 0.002 <0.0



Photograph (1) buccal smear showed Normal cell.X40. Wet fixed smear with H&E stain.



Photograph (2) buccal smear showed Inflammatory cell.X40. Wet fixed smear with H&E stain.



Photograph (3) buccal smear showed malignant cell.X40. Wet fixed smear with H&E stain.

Discussion

This study uses wet and dry H&E stains to examine the cytological alterations in buccal swab among hormone replacement therapy in 50 Sudanese women living in Khartoum State. Increases in the nuclear-cytoplasmic ratio have been found to be one of the primary indicators of premalignant and malignant lesions in microscopic studies. On the other hand, it is claimed that those receiving cancer treatment have worse dental health.

The pathology of buccal smears prepared by the wet approach was malignant (56%), inflammatory (34%), and normal (10%), as indicated in table (6). The dry method produced the same results. There were no appreciable variations in the pathology of the wet and dry-prepared buccal smears ($P=0.14>0.05$). Table (7) displays the corresponding results for the wet method and the dry method for buccal cytology affinity: excellent (40%), very good (28%), good (20%), acceptable (8%) and bad (4%) respectively. There is no discernible difference between the wet and dry techniques' affinity for the buccal smears ($P= 0.11>0.05$). In a research by Volpe et al. (2011), postmenopausal women experiencing mouth discomfort were given conjugated estrogens.

They discovered that HTR relieved both subjective and objective symptoms in more than half of the patients. Forabosco, et al. (2012).[30] investigated the effect of HTR on symptoms of mouth discomfort in postmenopausal women and concluded that oral discomfort may be associated with steroid hormone withdrawal only in some postmenopausal women, and that estrogen treatment may enhance the clinical picture in this group of women alone. Patil, et al. (2012).

Gynecologists in the Bagalkot area of Karnataka state were assessed for their awareness of the influence of female sex hormone on dental health. The majority were aware of the role of hormones on dental health throughout a woman's life. Gynecologists working at medical colleges were more aware than private practitioners. The authors stated that there is a need to educate caregivers about oral health. The identification of estrogen receptors using immunohistochemistry may aid in determining which patients would benefit from HTR. Table (8) shows that malignant pathology was found in 7.7%, 47%, and 95% of buccal smears from patients who had been on hormone therapy for <3 years, 3-9 years, and >9 years, respectively. There were significant differences in the pathology of buccal smears based on the duration of use. A questionnaire was used to explore the prevalence of self-assessed symptoms of painful mouth and dry mouth in 3173 menopausal women. They discovered that pain and dryness of the mouth were linked to climacteric symptoms in general, and that using HTR did not prevent or ameliorate symptoms. As indicated in table (9) the malignant pathology of buccal smears generated by wet and dry reported in 20%, 21.1%, and 88.5% of the smears obtained from patients who utilized hormone therapy at a frequency of one, two, and three times per day, respectively. There were significant changes in buccal smear pathology based on the frequency of hormone medication use per day ($P = 0.002 < 0.05$). Eliasson et al. (2003) Estriol was examined for its impact in enhancing saliva flow and the change in buffer capacity of saliva from minor salivary glands in 18 postmenopausal women (61-76 years). HTR caused a considerable increase in saliva flow, and symptoms of dry mouth decreased. Leiomola-Virtanen et al. (2000) studied the effects of HTR on saliva composition in 19 postmenopausal and 8 premenopausal women. They discovered that the proteins, immunoglobulin, and salivary peroxidase in both groups are estrogen-dependent.

5. CONCLUSIONS AND RECOMMENDATION

The pathology of wet and dry buccal smears revealed the highest level of malignancy, followed by inflammation and normal. There are no substantial pathological changes between wet and dry buccal smears. Both wet and dry methods produced buccal cytology with excellent higher affinity, followed by very good, good, acceptable, and horrible. The wet and dry techniques have the same affinity for buccal smears. The duration of hormone therapy administration was linked to significant changes in malignant pathology observed in buccal smears. The malignant pathology in buccal smears varied significantly according on the daily frequency of hormone medication use.

In the long run, women receiving hormone replacement therapy need close monitoring of their oral cavity and prompt treatment of any late problems after radiation therapy. Assessing the patient's psychological well-being is also important, especially in cases of depression. This is important because antidepressant drugs will lessen the somatization of pain and aid to relieve depression. Mucositis rarely poses a serious risk to life, but it can have a major impact on how well cancer treatments work.

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