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Gender effect on oral mucosa epithelium in blood disease patients

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ORIGINAL STUDY

Sex Effect on Oral Mucosa Epithelium in Blood Disease Patients

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Abstract

Purpose: To evaluate early detection of blood disease could be obtained by cytology. **Patients and methods:** A total of 82 patients, 41 females and 41 males with blood diseases including malignancies were selected for this study. Oral exfoliated cytology was used to detect changes in the oral mucosa using Gemisa stain. A microscopical examination was performed to detect three main cytological parameters which were cell shedding clusters, binucluated cells, and inflammatory background. **Results:** The highest frequency in the female group was with acute lymphoblastic leukemia 30.2% and the most affected age group was (21−30) counting 12.1% while in the male group was also acute lymphoblastic leukemia 42.9% and the affected age group was (11−20) counting 11.9%. For the cytological changes, in the female group; 100% of the cell cluster was seen in pancytopenia, pericardial infection, and multiple myeloma. While in the male group; 100% of cell clusters were seen in non-Hodjken lymphoma, aplastic anemia, pancytopenia, and splenectomy. Binucleuos feature was in the female group as 100% show binucleation in acute myloid leukemia, pancytopenia, hypoplasia anemia, and pericardial infection, while in the male group; 100% of pancytopenia, Burkitt lymphoma, and mycosis. For inflammatory background; 100% was seen in pancytopenia and pericardial infection female group. There was a significant correlation between cluster and binucleation in the male group, while there were highly significant correlations between binucleation and inflammatory background in the female group. **Conclusions:** Blood diseases affects oral mucosal epithelium and using oral cytology could be very helpful for early detection of these diseases that could be lifesaving.

Keywords: Binucleus, Cytology, Inflammation, Leukemia

1. Introduction

The blood diseases; in most cases, have a direct effect on many organs and tissue due to direct communication between blood vessels and the tissue. Oral mucosa represents a site of indirect manifestations of blood diseases which show signs and symptoms that could be useful in the early diagnosis of these diseases.

These effects vary from simple epithelial changes to deep mucosal and submucosal alterations and pathological conditions that may be related to metastatic conditions [1].

Using cytological examination in the diagnosis of oral lesions including precancerous and cancerous lesions may be one of the aiding methods in the early detection of these lesions that could affect the prognosis of these serious conditions [2−5].

Epithelial changes in oral diseases; including systemic manifestations which may be seen as various criteria like exfoliation in cell clump organization with different forms like clusters, papillary, sheets and glandular [6].

Other cellular changes including cytoplasmic and nuclear changes [7] which indicated environmental and genetic alterations that affect cell proliferation and maturation [5] like binucleation which indicated abnormal mitosis due to cleavage furrow or fail cytokines and multipolar spindles and mostly seen in cancer cases [8].

The early diagnosis of systemic malignancy through oral cytology could be useful metastases or in case of blood premalignant and malignant conditions like acute lymphocytic leukemia and lymphoma [7−9].

Biopsy, fine needle aspiration, and oral brush cytology are the main tools used for early diagnosis

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of premalignant and malignant oral lesions that had been studied in many kinds literatures.

The aim of this study was using oral brush cytology for early detection of oral lesions associated with blood diseases and this study represents the first one in Iraq.

2. Patients and methods

1-Patients: 82 patient samples were recruited in this study as 41 female and 41 male. The sample collected from patients attending the hematological department 7th floor in Baghdad Teaching Hospital, Baghdad-Iraq in the duration between April- and June 2018.

Complete medical history was obtained from the patient's table charts for correlation study including age, sex, blood test and other.

Different blood diseases were selected for comparison between them. These diseases are illustrated in Table 1 which includes malignant and nonmalignant blood descries.

The patients with malignancies were diagnosed and under treatment with chemotherapy according to their severity.

Those patients were free from observed clinical oral symptoms that may accompany hematological disorders. Besides; most of the patients were under prophylactic treatment for oral infection like

Table 1. Sex distribution of patient's sample within age groups.

Type of disease	Sex	Age distribution								Total
		1–10	11–20	21–30	31–40	41–50	51–60	61–70	>70	
ALL	Female	–	3 (9.1%)	4 (12.1%)	1 (3%)	–	–	1 (3%)	1 (3%)	10 (30.2%)
	Male	–	5 (11.9%)	4 (9.5%)	4 (9.5%)	2 (4.8%)	2 (4.8%)	–	1 (2.4%)	18 (42.9)
AML	Female	–	2 (6.1%)	1 (3%)	2 (6.1%)	1 (3%)	1 (3%)	1 (3%)	–	8 (20.2%)
	Male	–	2 (4.8%)	1 (2.4%)	1 (2.4%)	1 (2.4%)	–	–	–	5 (12%)
NHL	Female	1 (3%)	1 (3%)	2 (6.1%)	–	1 (3%)	–	1 (3%)	–	6 (18.2%)
	Male	–	2 (4.8%)	1 (2.4%)	–		1 (2.4%)	–	–	4 (9.5%)
HD	Female	–	–	1 (3%)	–	1 (3%)	–	–	–	2 (6%)
	Male	–	–	–	–	–	–	–	–	–
MM	Female	–	–	–	–	–	1 (3%)	–	–	1 (3%)
	Male	–	–	–	–	–	–	–	–	–
AA	Female	–	–	–	1 (3%)	–	1 (3%)	–	–	2 (6%)
	Male	–	1 (2.4%)	–	–	–	–	–	–	1 (2.4%)
Panacytopenia	Female	–	–	1 (3%)	–	1 (3%)	–	–	–	2 (6%)
	Male	–	–	–	–	–	–	1 (2.4%)	–	1 (3%)
Hypoplastic aneamia	Female	1 (3%)	–	–	–	–	–	–	–	1 (3%)
	Male	–	–	–	–	–	–	–	–	–
Moderate pericardial infection	Female	–	–	–	–	1 (2.4%)	–	–	–	1 (2.4%)
	Male	–	–	–	–		–	–	–	–
Spleenctomy	Female	–	–	–	–	–	–	–	–	–
	Male	–	–	–	1 (2.4%)	–	–	–	–	1 (2.4%)
Burikt lymph	Female	–	–	–	–	–	–	–	–	–
	Male	–	2 (4.8%)	–	–	–	–	–	–	2 (4.8%)
Lytic bone marrow	Female	–	–	–	–	–	–	–	–	–
	Male	–	–	–	1 (2.4%)	–	–	1 (2.4%)	–	2 (4.8%)
Blast marrow	Female	–	–	–	–	–	–	–	–	–
	Male	–	–	–	1 (2.4%)	–	–	–	–	1 (2.4%)
MD	Female	–	–	–	–	–	–	–	–	–
	Male	–	–	1 (2.4%)	–	–	–	1 (2.4%)	–	2 (4.8%)
Bone marrow tumor	Female	–	–	–	–	–	–	–	–	–
	Male	–	–	–	–	1 (2.4%)	–	–	–	1 (2.4%)
MTA	Female	–	–	–	–	–	–	–	–	–
	Male	–	–	–	1 (2.4%)	–	–	–	–	1 (2.4%)
Platelets reduction	Female	–	–	–	–	–	–	–	–	–
	Male	–	1 (2.4%)	–	–	–	–	–	–	1 (2.4%)
Mycosis infection	Female	–	–	–	–	–	–	–	–	–
	Male	–	–	–	–	–	–	1 (2.4%)	–	1 (2.4%)
Leukopenia	Female	–	–	–	–	–	–	–	–	–
	Male	–	–	1 (2.4%)	–	–	–	–	–	1 (2.4%)

candidiasis by using proper tooth brushing and mouth wash.

2.1. Sample collection for cytology

Oral cytology brush was used to get cytological smear from the epithelial layer from the labial gingiva of the lower anterior teeth region of the patients. Fixed by using 95% ethyl alcohol and stained by Gemisa stain to view the cellular changes under the light microscope (Olympus/Italy).

The observed cells were squamous cell epithelium of the labial gingival mucosa that exfoliated by brush scraping.

2.2. Microscopical examination

The slides were analyzed using a digital optical microscope (Micros, Austria) at a magnification of 1000x (oil immersed objective lens = 100x with eyepiece = 10x). The slides were analyzed using a digital optical microscope (Micros, Austria) at a magnification of 1000x (oil immersed objective lens = 100x with eyepiece = 10x).

The observed structures that were used to compare cell clusters, binucleus, and inflammatory background.

2.3. Statistical analysis

SSPS Version 22 was used for statistical analysis. Frequencies, *t*-test, ANOVA, Person correlation tests were used to analyze the data collected.

2.4. Ethical approval

The data were collected after the approval of the ethical committee in the Department of oral pathology/College of Dentistry/Mustansiriyah university in April-2018. In addition to proper agreements from Baghdad Teaching hospital-Iraq.

3. Results

Clinical observations: each group of males and females had a different distribution of hematological disorders as seen in the Table 1. The highest frequency was seen in the female group with acute lymphoblastic leukemia (ALL) 10 (30.2%) and the most age group were (21–30) counting 4 (12.1%) while in male group was also ALL 18 (42.9%) and age group (11–20) counting 5 (11.9%).

3.1. Cytological changes

The results are summarized in Tables 2–4.

3.1.1. Cell cluster

In the female group; pancytopenia, pericardial infection and multiple myeloma 100% of the cell

Table 3. Person's correlations between groups.

Parameters	*P* value	Significance
Binucleus1 x inflammatory1	0.454 (0.008)[b]	HS
Cluster1 X Binucleus 1	0.316 (0.041)[a]	S

[a] Correlation is significant at the 0.05 level (2-tailed).
[b] Correlation is significant at the 0.01 level (2-tailed).

Table 2. Frequency distribution of cytological parameters among groups.

Type of disease	Cell cluster		Bi-nucleation		Inflammatory background	
	Female	male	Female	Male	Female	Male
ALL	5 (50%)	13 (72.2%)	3 (30%)	8 (44.4%)	4 (40%)	5 (27.7%)
AML	3)337.5%)	3 (60%)	3 (37.5%)	1 (20%)	1 (12.5%)	5 (100%)
NHL	5 (83.3%)	4 (100%)	3 (50%)	1 (25%)	1 (16.7%)	–
HD	1 (50%)	–	1 (50%)	–	–	–
MM	1 (100%)	–	1 (100%)	–	–	–
AA	1 (50%)	1 (100%)	–	–	–	1 (100%)
Panacytopenia	2 (100%)	1 (100%)	2 (100%)	1 (100%)	2 (100%)	–
Hypoplastic anemia	–	–	1 (100%)	–	–	–
Moderate pericardial infection	1 (100%)	–	1 (100%)	–	1 (100%)	–
Spleenctomy	–	1 (100%)	–	–	–	–
Burikt lymph	–	2 (100%)	–	2 (100%)	–	1 (50%)
Lytic bone marrow	–	–	–	–	–	–
Blast marrow	–	1 (100%)	–	–	–	1 (100%)
MD	–	1 (50%)	–	–	–	–
Bone marrow tumor	–	–	–	–	–	–
MTA	–	1 (100%)	–	–	–	–
Platelets reduction	–	–	–	–	–	–
Mycosis infection	–	–	–	1 (100%)	–	–
leukopenia	–	–	–	–	–	–

Table 4. Analysis of variance test between all tested male and female group.

ANOVA Summary					
Source	Degrees of Freedom DF	Sum of Squares SS	Mean Square MS	F-Stat	*P* Value
Between Groups	5	6.1004	1.2201	5.5991	0.0001
Within Groups	252	54.9123	0.2179		
Total:	257	61.0127			

cluster, while (Non-Hodgkin lymphoma showed 83% and Acute lymphocytic leukemia, aplastic anemia Hodgkin disease showed 50% and 37.5% in Acute myeloid leukemia.

In the male group; 100% of cell clusters were seen in NHL, AA, pancytopenia, splenectomy, Burkitt lymphoma, blast marrow and MTA while 72.2% in ALL and 60% in AML. MD; showed 50% of the cell clusters (Fig. 1).

3.1.2. Binucleuos

In the female group; 100% show binucleation in AMM, pancytopenia, hypoplasia anemia and pericardial infection, while 50% in NHL and HD. While 37.5% in AML and 30% in ALL.

In the male group; 100% of pancytopenia and Burkitt lymphoma, and mycosis; while 44.4% in ALL. 20% in AML and 25% in NHL (Fig. 2).

3.1.3. Inflammatory background

In the female group; 100% was seen in pancytopenia and pericardial infection, while 40% in ALL, 16.7% in NHL and 12.5% in AML.

100% in AML, ALL and blast marrow, while 50% in Burkitt lymphoma, while 27.7 in ALL (Fig. 3).

Pearson correlations showed a significant correlation between cluster and binucleation in the male group, while there were highly significant correlations between binucleation and inflammatory background in the female group as shown in Table 3.

While in ANOVA tests was found a significant relation with F-value 5.59. Between male and female group as shown in Table 4.

Data summary was shown in Table 5.

Fig. 2. Binucleated epithelium cell of oral mucosa in female patient with AML.X 1000.

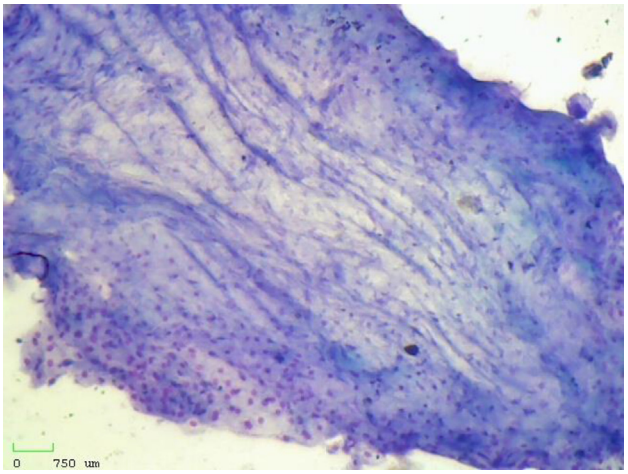

Fig. 1. Cluster shedding of the oral epithelium in a male patient with panacytopenia. X 20.

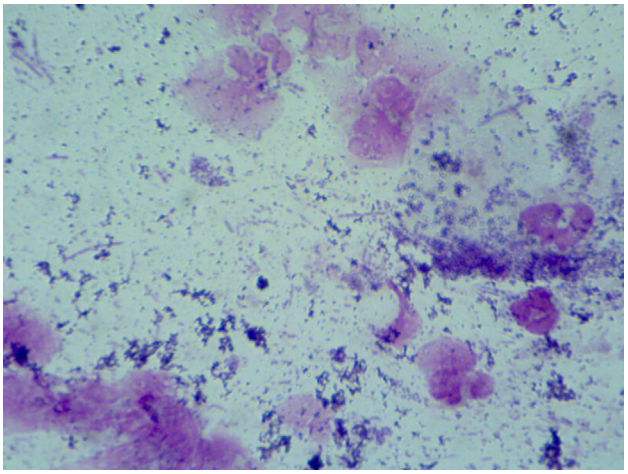

Fig. 3. Inflammatory background of the oral epithelium in a male patient with acute lymphoblastic leukemia.X 1000.

Table 5. Data summary.

Groups	N	Mean	Std. Dev.	Std. Error
Cluster (female)	33	0.5758	0.5019	0.0874
Cluster (male)	42	0.6429	0.485	0.0748
Binucluation (female)	33	0.3636	0.4885	0.085
Binucluation (male)	42	0.3333	0.4771	0.0736
Inflammatory Background (female)	66	0.2424	0.4318	0.0532
Inflammatory Background (male)	42	0.2619	0.445	0.0687

4. Discussion

The oral cytology represents a mirror of many systemic conditions including malignant diseases and the early diagnosis of these conditions and diseases may be represent a lifesaving aid that could be obtained by simple examinations and tests. Oral cytology brush could be one of these equipment's that are used to discover premalignant and malignant oral lesions in addition to other oral lesions [10].

Metastatic secondary tumors could be also found in the oral cavity especially from lung, breast, and tests which metastases to the jaw bone, mainly but there metastasis to the oral mucosa could be very rare and their discovery could be misdiagnosed with primary oral malignancy [11,12].

While blood diseases, including blood dyscrasia and malignancy, were manifested in the oral cavity mostly due to their direct or indirect manifestation in the oral mucosa. But the early diagnosis of these diseases through oral mucosa changes has always represented a challenge for dentists and even physician [13–15].

Using an oral cytology brush for early detection of blood diseases including serious conditions had not been studied primarily.

In this study, there was an obvious relationship between blood diseases and oral mucosal changes through certain cytological parameters that related mainly to malignant changes like cell cluster shedding, binucleation and inflammatory background.

For Cell cluster shedding ALL showed a higher cell cluster shedding in the male group while AML was higher in the female group which indicates the effect of sex on both types of leukemia, which in turn affects the shedding of the epithelium in the form of cluster. These cells mostly were nucleated which indicated the shedding was not a normal dead epithelial cells, but the shedding of the deeper epithelium layers that may reflect chronic inflammatory conditions [16,17]. The effect of sex was obvious on the cluster cell shedding among hematological diseases that was the predominance higher percentage than female. This may be due to the female sex hormones, which have their effect on the epithelium [18,19].

For binucleated cells; the cells can arise in a culture as a result of an incomplete process of cell division, i.e. karyokinesis with incomplete cytokinesis or as a result of the mitotic division of a pre-existent binucluated cells. The mitotic division of a binucluated cells can give rise to different types of daughter cells [20,21].

Most of the female patients showed marked binucleation of the shed the oral epithelium with less extent for male patients except for ALL.

This may be related to x-linked genes that this type of feature of cytology. Age-Dependent Inclusion of Sex Chromosomes in lymphocyte micronuclei of man [22].

For the inflammatory background most of the hematological diseases including malignancies affect the inflammation process through the defect on defense cells, but due to that the patients in our study group were under chemotherapy treatment or other types of treatment. Chronic inflammation has been linked to various steps involved in tumorgenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis [10]. But the most affected patients that showed inflammatory background were related to the disease's effect directly white blood cell like ALL, AML. This agreed with Hirshberg suggested that inflammation plays an important role in attracting metastatic cells to the gingiva [23], Again, there were variations between the males and females patients.

The correlations between the cytological parameters showed marked relation between the binucleation and two other parameters inflammatory backgrounds and the cluster shedding in females group only which indicated the other effects of sex on cytological expression of the diseases [19].

4.1. Conclusions

Cytology could be useful for early diagnosis of different systemic diseases including hematological disorders especially blood malignancy through oral mucosal smears which give an indications for abnormalities by certain parameters like epithelial shedding, binucleation and inflammatory background of oral epithelium.

4.2. Recommendations

It is recommended that further studies would be needed for each blood disease and there correlation with oral cytology.

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Conflicts of interest

The author declare that there is no conflict of interest.

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