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### RESEARCH ARTICLE

#### ASSESSMENT OF CYTO-MORPHOLOGICAL CHANGES IN EPITHELIAL ORAL MUCOSA AMONG SICKLE CELL ANEMIA PATIENTS ATTENDING MANAGIL PEDIATRIC TEACHING HOSPITAL, GEZIRA STATE ,SUDAN (2017).

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#### Abstract

Sickle cell anemia (SCA) is an autosomal recessive genetic disorder, characterized by chronic hemolytic anemia and affect oral soft and hard tissues, the clinical finding related to anemia affecting of oral mucosa such as paleness oral mucosa, some cysts and apparently oral ulcers or ischaemic ulcers due to occlusion of some mandibular vessels as a result of reduction of blood supply this cross sectional - study conducted in Managil Pediatric Teaching Hospital, about 80 smears were collected from buccal mucosa of sickle cells anemia patients there was no significant difference were found of SCA patients according to the gender regarding Cytological Atypia , there was significant correlation between the Age and Cytological Atypia in SCA patients and finally the Cytological Atypia of oral mucosa in SCA patients were detected in 87% of SCA patients 18% were mild (+) , 42 % were moderate(++) and 27% were marked (+++).

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#### Introduction:

Sickle cell anemia (SCA) characterize by homozygous hemoglobin S, the clinical finding related to anemia affecting mainly the oral mucosa (1). There have been four publications in the world literature describing some cases of a rare complication of SCA , in which mandibular pain were followed by numbness of the lip and chin and that was explained by blockage of the mandibular vessels (2) Studies using exfoliatives cytology or liquid-based exfoliative cytology have demonstrated that the nuclear and cytoplasmic areas of the epithelial cells in oral mucosa may be modified due to systemic conditions such as anemia., sickle cell anemia a common inherited disorder shows a wide distribution in the Sudan they common in Western Sudan(Darfur) with a prevalence rate of 18% to 30.4%. (3)(4). Sickle Cell Disease was characterized by chronic intravascular and extra vascular haemolysis Sickling-induced membrane fragmentation and complement-mediated lysis cause intravascular destruction of red cells. Membrane damage also leads to extra vascular haemolysis through entrapment of poorly deformable cells or uptake by macrophages, patients have greatly expanded bone marrow space, but the serum erythropoietin level is lower than expected for the extent of anaemia owing to decreased oxygen affinity of HbS.

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**Material and Method:****Materials and equipments were utilized in this study:**

0.9% sodium chloride, gloves, Flat wooden spatula, Slides, 95% ethyl alcohol, Marker for labeling, Slides holder, Coplin jars, Filter paper, 70% alcohol, 80% alcohol, 100% alcohol, Harris Hematoxylin, DW, tap water, Papanicolaou stain (orange G-6 and Eosin Azoure 50), Cover slips, Wooden stick, DPX, Slides box, Oil, light Microscope.

**Sampling Procedures**

Cytological smears were collected from buccal mucosa after patients washed a mouth with 0.9% sodium chloride solution for five minutes and used a flat wooden spatula to scrape cells immediately, while they were wet smears done. They labeled and fixed by dipping in 95% alcohol for 15 minutes to process by pap stain, (The label were keeping away until all the smears were examined).

**Staining Procedures:**

The study was used Papanicolaou stain.

**Method:**

The study used exfoliative cytology (indirect exfoliative cytology buccal smear) to assess cyto-morphological changes, the data and smears collected after filing consent and questionnaire form, the smears were treated according to Papanicolaou method.

Place slides 95%, 80% Alcohol, 70% Alcohol and D.W for 8 minutes, rinse in running tap water for 2 minutes, Stain in Harris Hematoxylin stain 3 minutes, wash in tap water until clear, blue slides in Scott tap water substitute for 30 seconds, wash in tap water for 30 seconds, dehydrate in 70% ethyl Alcohol; 10 dips, dehydrate in 95% ethyl Alcohol, 10 dips, stain in Papanicolaou stain, OG-6 for 3 minutes, rinse in two changes of 95% ethyl Alcohol, stain in Papanicolaou stain, EA-36, EA-50 or EA-65 of choice for 3 minutes, dehydrate in two changes each of 95% and 100% ethyl alcohol and clear in three changes of xylene, cover slip with mounting medium. (6).

**Interpretation:**

Cyto-morphological changes that regard as atypia when the nuclear enlargement were associated with increased nuclear-cytoplasmic ratio, hyperchromasia, with moderately prominent nucleation and bi or multinucleation, increased cellular polymorphism variations in size and/or shape of cells and nuclei.

**Cytological Diagnosis and Microscopic Examination:**

The criteria followed for the detection of the cytological changes was detect the presence of inflammatory cells and/or. Hyperchromasia, were assessed, increased N/C ratio, in the form of enlarged nuclei was found, cellular pleomorphism different shape and size and, finally multinucleation, were detected and assessed. Each change of the above characteristic was given cytological Atypia score as: (0) normal, (+) mild (++) moderate and (+++) marked. According to the severity of the change encountered (7).

**Scoring Method of Atypia:**

The score of Atypia was developed according to the cytological parameters such as hyperplasia, and nuclear features. Scored according to the number of criteria as (0) Normal, mild (1-2), moderate (3-4) and severe or marked (5-8) (8).

**Statistical Analysis:**

All data were tabulated and statistical tests were performed with SPSS for Windows version 15.0, Chi-square test was used to verify if there was difference in morphological characterization of oral smears according to age. Differences were considered statistically significant when  $P = 0.05$ .

**Results:**

Results showed the mean age of male was 8.03 years and mean age of female was 7.60 years, they were analyzed by Statistical Package for Social Science Computer Program version 15 and Microsoft Office Excel Computer Program, there was no significant difference were found in the Cytological Atypia of SCA between

male and female (p. value 0.9 ) , there was significant correlation between the Age and Cytological Atypia in SCA patients (p. value 0.05).

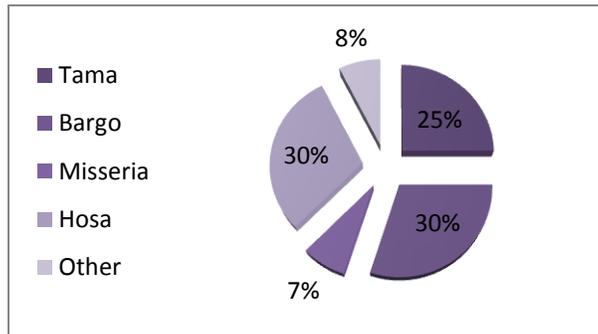


Figure 1:-Distribution of SCA patients according to Tribe

Table 1: Correlation between ages in the frequency of cytological Atypia of SCA patients by used cross tabulation test.

Age	Normal F	Mild F	Moderate F	Marked F	Total F	P-value
>year	4	2	2	0	8	0.05
1-4year	6	4	6	0	16	
5-8year	0	8	8	6	22	
9-13year	0	0	12	12	24	
14-17year	0	0	6	4	10	
Total	10	14	34	22	80	

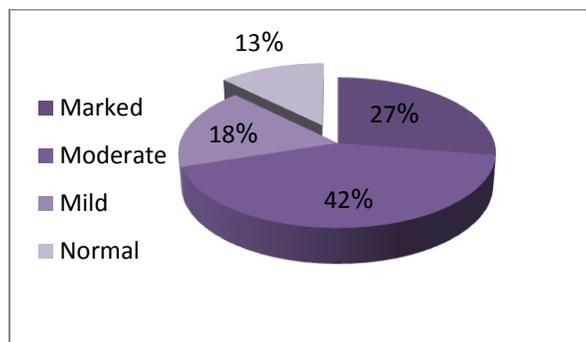
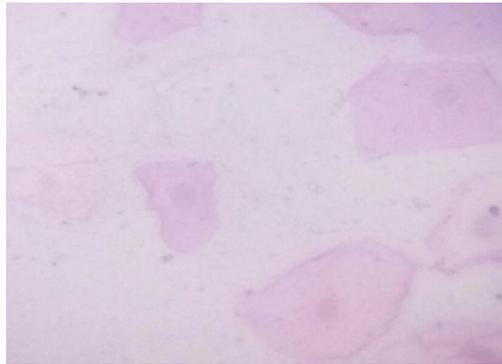


Figure 2:-percentage of cytological Atypia criteria in SCA patients

Table 2:Correlation between sex in the frequency of cytological Atypia of SCA patients by used cross tabulation test

Sex	Normal F	Mild F	Moderate F	Marked F	Total F	P-value
Male	6	8	16	10	40	0.922
Female	4	6	18	12	40	
Total	10	14	34	22	80	

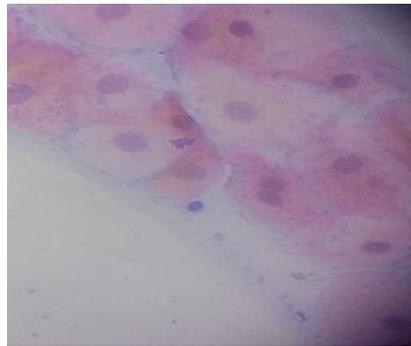
**Figure 3:-**Cytological smear of SCA patient appear cellular polymorphism and increased nuclear- cytoplasmic ratio(magnification 40)



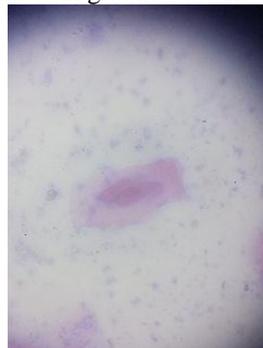
**Figure 4:-**chronic inflammation change of patient with sickle cell anemia inflammatory cells ( neutrophil and macrophage) (Histocyte) and have backgound bacillus



**Figure 5:**Oral mucosal cells in SCA patient appear Cellular and Multinucliation and hyperkeratosis (magnification40)



**Figure 6:** Cytological smears appear increased nuclear - cytoplasmic ratio and prominent nucleation and irregular nuclear borders and back ground bacteria (magnification 40)



**Discussion:**

cytological Atypia detected in 87% of SCA patients ,18% were mild (+) ,42 % were moderate (++) and 27% were marked ( +++), there was no significant difference were found in the Cytological Atypia of SCA between male and female (p.value 0.9) , there was significant correlation between the Age and Cytological Atypia in SCA patients (p.value 0.05)

**Conclusion:**

1. The cyto-morphological changes detected among patients with sickle cell anaemia
2. There was no significant difference were found in the Cytological Atypia of SCA regarding the gender, there was significant correlation between the age and cytological Atypia in SCA patients.

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