

## Micronucleus Frequencies in Buccal Cells from Patients with Sick Cell Anaemia

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### Abstract:

Presence and frequencies of micronuclei in buccal cells from patients with sickle cell anaemia (SCA) were detected. The aim of this study was to investigate the genome stability in patients and carriers of single gene for sickle cell disease. Buccal swabs were collected from 12 patients with SCA [(6 females and 6 males (Group 1)] and from 8 individuals carrying single gene of SCA (Group2). Ten healthy individuals matching in age and gender were also included in this assay as controls (Group3). Numbers of cells with micronucleus and un-nucleated cells were counted in all the three groups as parameters for the evaluation of genome stability. Significant number (2-12/1000 cells) of buccal cells with micronuclei was seen in patient's cells.

However; one of the individuals with SC trait showed cells with MN. Control groups have very few cells with MN 1-3/1000 cells. Karyolytic (un-nucleated) cell number in group 1 was 2.5 times more than in group 2 and 3 respectively compared to those of the controls. These results suggest that patients with sickle cell anaemia have unstable genome when both causative mutant genes are present. Presence of apoptotic cells is solely indicating their somatic tissue damage as a result of the disease.

### Introduction:

Sickle Cell Anaemia (SCA) is an inherited blood disorder, characterized by episodes of pain, chronic haemolytic anaemia and severe infections. Sickle cell anaemia (SCA) is an autosomal recessive disease caused by a point mutation in the haemoglobin beta gene (HBB) found on chromosome 11 p 15.5. A mutation in HBB results in the product of structurally abnormal haemoglobin, called Hbs. Under certain conditions, like low oxygen level or high haemoglobin concentration, in individuals who are homozygous for Hbs the abnormal Hbs cluster together which then disorder the RBCs into sickle shapes. These deformed and rigid RBCs become trapped in small blood vessels and block them, producing pain and eventually damaging organs. This can result in hand-foot syndrome, fatigue, paleness, and shortness of breath, pain that occurs unpredictably in any body organ or joint. SCA patients also suffer from yellowish skin and eyes, delayed growth and puberty in children. (1, 2, 3). Individuals who possess one copy of the normal beta globin gene (HBB) and one

copy of the sickle variant (Hbs) are referred to as having sickle cell trait, but these individuals do not express symptoms of sickle cell disease (4).

Carrier frequency of sickle cell anaemia varies significantly around the world. There is a high prevalence of SCA in Oman. Ministry of Health in Oman reported that around 10% of the Omani population carry genes for sickle cell anaemia (5) when total Omani population in 2007 was 2,743,499 individuals (6). On the other hand, birth prevalence of SCA was reported to be 2-2.7/1000 (7).

Consanguineous marriages are known to increase the risk of expression of autosomal recessive conditions in the offspring, particularly the rare disorders (8). The consanguinity rate in Oman was reported to be high (9) which is one of the reasons why Oman has higher rate of SCA.

A micronucleus (MN) is formed during the metaphase/anaphase transition of mitosis. It may rise from a whole lagging chromosome (a eugenic event leading to chromosome loss) or an acentric chromosome fragment detaching from a chromosome

after breakage (Clastogenic event) which do not integrate in the daughter nuclei (10).

The buccal cell (BC) micronucleus (MN) assay, first proposed by Stich et al (10, 11), is useful as a biomarker of genetic damage caused by life-style habits (e.g. smoking consumption and/or alcohol micronutrient deficiency) and of exposure to environmental pollutants as well as in patients with inherited genetic defects in DNA repair (12).

The objective of this study is to assay the genomes' stability in patients with sickle cell anaemia by a micronucleus assay in buccal mucosa cells.

## Materials and Methods:

### Study population

Twenty clinically diagnosed sickle cell anaemia patients were screened in this study. Twelve of them (6 females and 6 males) were having Hbs (Group 1) and eight had SC traits (2 females and 4 males) (Group 2). Their ages ranged between 18-24 years, and living under similar socioeconomic conditions. Ten controls of the same age group (18-25 years)(Group 3) and gender volunteered at the time of testing.

### Cell sampling and preparation

Buccal cells (BC) were sampled from the consented volunteers by following method of Tolbert et al (13) and Belien et al (14), after modifications. Prior to BC collection the mouth of each volunteered was rinsed thoroughly with water to remove any unwanted debris. Small-cotton headed sterilized sticks were rotated several times in a circular motion against the inside lining of the cheek. The head of the stick (cotton swap) was swapped on clean slides to make smear. Slides were then fixed with methanol for 10 minutes, rinsed with distilled water, and stained with

Giemsa for 20 minutes, rinsed with buffer (pH 6.8) and air dried.

### 1. Cell scoring

Normal differentiated cells (Figure 1 A); these cells have a uniformly stained nucleus that is usually oval or round in shape. No other DNA-containing structures apart from the nucleus were observed in these cells.

### 2. Cells with micronucleus (Figure 1B-2B)

These cells were characterized by the presence of a main nucleus and one-three smaller nuclei called micronuclei (MNs) (Figure 1B-3B). The MNs were usually round or oval in shape and their diameter ranged between 1/3 or 1/10, the diameter of the main nucleus.

### 3. Karyolytic cells

In these cells, the nucleus is completely depleted of DNA and is appeared not to have a nucleus(Figure 1.C).

### Scoring method

Initially, more than two thousand cells were scored per subject for all the various cell types. These consisted of normal cells, cells with micronucleus, as well as cells without nucleus.

### Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the significance of the cellular parameters measured between controls, SC traits and sickle cell anaemia patients.

## Results:

The photographs for cells with micronuclei and cells without nucleus (Karyolytic cells) from sickle cell patients and carriers are presented in figures 1 A, B, and C and Figures 2A and B.

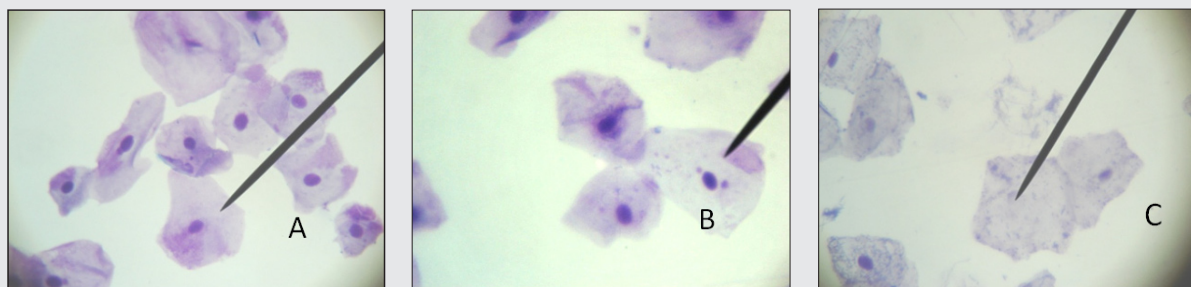


Figure1: Images showing (A) normal buccal cell. (B): buccal cell with micronuclei (MN). (C): un-nucleated buccal cell.

Cells with micronuclei were significantly ( $P < 0.05$ ) more in patients with SCA group (MN 4-2/1000 cells), compared to controls (0.65MN/1000 cells) with a range of 1-11 cells/1000 cells. However, only one carrier of SC trait out of six had micronucleus-containing cells (Figure 3).

Karyolitic cells were significantly higher in number

in patients with SCA (about 3.5 times more) than those in the control individuals, while SC traits have doubled the base line level (1.55% vs. 0.7%) in the controls (Figures4). There were no significant differences, based on the gender, between the control and patient groups.

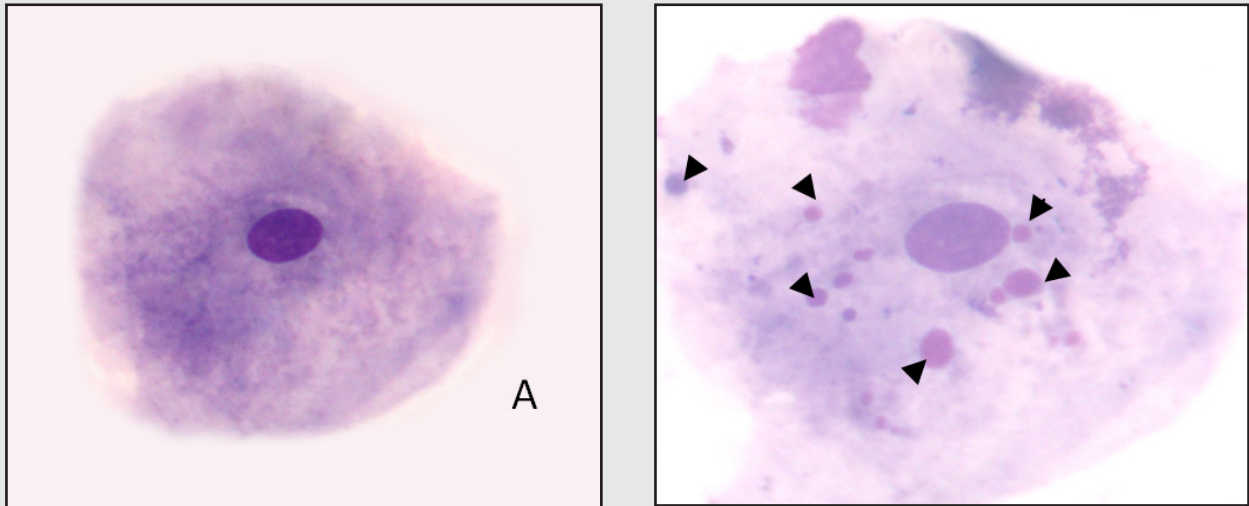


Figure2: Images showing (A) normal buccal cell. (B): buccal cell with 11 micronuclei (arrows) isolated from patients with sickle cell anemia

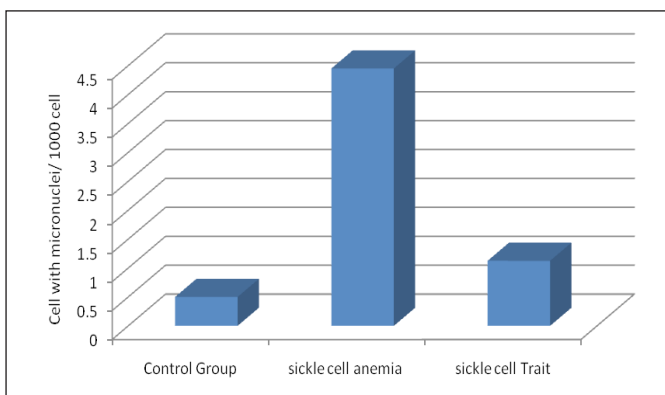


Figure 3: Frequencies of buccal cells with micronuclei from patients with Sickled disease.

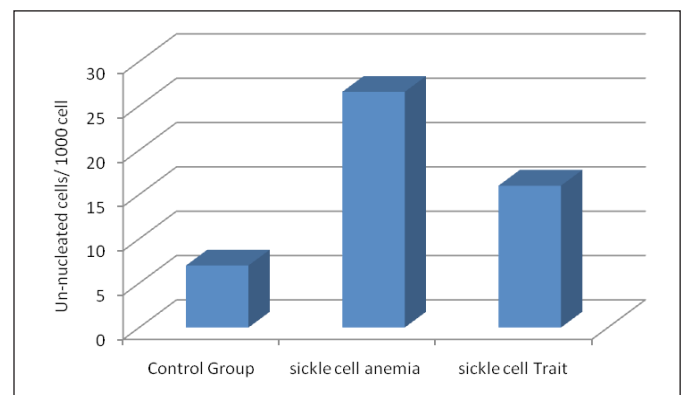


Figure 4: Frequencies of un-nucleated buccal cells from patients with Sickled disease.

## Discussion:

Studies on the frequency of micronuclei existence in buccal cells from patients with sickle cell anaemia have not been reported so far. In order to contribute the understanding of the role of the different biomarkers and their relationship with the extremely variable clinical manifestation of sickle cells disease, we investigated the MNs frequency in buccal cells in sickle cell traits, sickle cell anaemia patients and controls. We found an increased frequency of micronuclei and percentage of karyolytic cells in these patients when compared with control subjects. Causes of the presence of micronuclei in buccal cells from SCA patients are unknown. It was reported that SCA patients are subjected to increased oxidative stress particularly during vaso-occlusive crises and acute chest pain. Another possible cause of oxidative stress in SCD is the high concentration of iron in the patients' plasma. The increase in oxidative stress could be a relevant risk factor for mutagenesis and carcinogenesis (15). Moreover, patients with sickle cell anaemia often are suffering from folic acid and vitamin B12 depletion because of their chronic haemolytic anaemia (16). Folic acid and B12 play a critical role in the prevention of chromosome breakage that leads to micronucleus formation. Both in vitro and in vivo studies with human cells clearly show that folate B12 deficiency causes expression of chromosomal fragile sites, chromosome breaks, excessive uracil in DNA, micronucleus formation and DNA hypermethylation (17, 18).

Micronucleus observed in buccal cells is not induced when the cells are at the epithelial surface but are formed in the basal layers (19).

There was no significant difference between individuals, carrying single sickle cell gene and

control groups in the presence of micronucleus in their buccal cells (Figure 3). These results may suggest that formation of micronucleus is highly associate with SCA severity i.e. presence of both SC genes. Such presence would lead to genome instability which resulted in micronucleus formation.

Karyolytic cells represent buccal cells with apoptosis. These cells, however, are considered as a biomarker in clinical trials of cancer therapy (20) as well as in patients with Alzheimer's disease who have higher frequency of MN in their buccal cells (21). The present results of the study showed that patients with sickle cell anima and the carriers have an elevated levels of un-nucleated cells, 3.5 or 2-times in SCA and traits, respectively compared to those of the control (figure 4).

The cells that appear to have no nucleus, are probably representing a very late stage in the cell death process, but this has not been conclusively proven.

These result might suggest that patients with sickle cell anaemia who develop this disease as a result of inheritance of single gene mutation from both parents having genome instability which is represented by the presence of micronuclei in the somatic cells, this is highly associated with gene dose.

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## تردد النويات الدقيقة في مرضى فقر الدم المنجلي

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### الخلاصة:

هدف هذه الدراسة الى الكشف عن استقرارية الجين لدى المرضى المصابين بفقر الدم المنجلي باستخدام تقنية فحص القوى المجهرية في الخلايا المبطنة للفم. تم جمع مسحات الفم من 20 مريضا انثى، عشرة منهم كانوا مصابين بالمرض وثمانية يحملون احدى الجينات الطافرة والذي لا يظهرون أعراض وعشرة من الأشخاص السليمين تطوعوا كمجموعة سيطرة. حضرت شرائح مجهرية من تلك المسحات وصبغه بصبغة كيمزا وفحصت الخلايا السليمة، الفاقدة للنواة وأخرى الحاملة للنوى المجهرية بعدد 2000 خلية لكل شخص. لوحظت زيادة معنوية في عدد الخلايا الحاوية على النوى المجهرية لدى المرضى حيث كان ترددها يعادل 8 أضعاف مما هو عليه في خلايا الأشخاص السليمين ومدى 2-12 نواة مجهرية/للخلية بينما كان المدى 1-3 نواة مجهرية/للخلية لدى السليمين. أما حاملي الجين الممرض فكان العدد قد أصبح الضعف. لقد أظهرت نتائج فحص الخلايا الفاقدة للنواة لدى فقر الدم المنجلي زيادة معنوية تساوي 5 أضعاف و3 أضعاف عند حاملي المرض، مقارنة بأعدادها لدى السليمين. تشير هذه النتائج الى عدم استقرارية جين مرضى فقر الدم المنجلي في الخلايا الجسمية وزيادة في عدد الخلايا المضمحلة.