

# Cytological Findings of Buccal Mucosa in Megaloblastic Anemia

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## ABSTRACT

Megaloblastic anemia is a common problem in Pakistan, that can lead to negative health outcomes with increasing age. Apart from the general effect due to the deficiencies of folate and vitamin B12 as observed in blood, bone marrow cells and epithelial cell surfaces, similar changes in oral epithelium have also been found. These cellular findings however have been limited to a very few studies and offers a good research scope for more studies to be conducted. Healthcare strategies that consider the impact of laboratory tests on the overall costs and quality of care should consider the advantages of including cytological findings of buccal mucosal scraps for evaluation of these patients and can serve as a prerequisite of diagnosing this disease.

## INTRODUCTION

Megaloblastic anemia (MGA) is a commonly occurring anemia, due to vitamin folate and vitamin B12 deficiency. It is characterized by the presence of large, structurally and visibly abnormal, immature red blood cells (megaloblasts)<sup>1</sup>. The usual presenting age in the developed world is infancy. But in the developing countries, like Pakistan, it can occur at any age. Megaloblastic anemia (MGA) can cause serious complications ranging from neuro-psychiatric signs, tachycardia, dyspnea and hyperpigmentation. With an increasing age it can further escort to negative health outcomes<sup>2</sup>.

Apart from the general effect due to the deficiencies of folate and vitamin B12 as observed in blood, bone marrow cells and epithelial cell surfaces, similar changes in oral epithelium have also been found. The usual presenting oral complaint of megaloblastic anemia is varying degree of mucosal bleeding, glossitis and ulcerations<sup>2-5</sup>.

### Epidemiology and etiology

Historically, there was a wide disparity in the occurrence of MGA in ethnic population around the world and amongst the Asians; its occurrence was common in South Asia<sup>6</sup>. However, during recent

years, it has become apparent that occurrence of MGA in all racial and ethnic groups is more common than was previously recognized.

In Pakistan, MGA has been observed to be prevalent in every age, which is an irony since Pakistan is an agricultural country and folate is abundant in the food especially green leafy vegetables<sup>3</sup>. There is a scant data available on the prevalence of MGA due to lack of reliable population based studies. However, the prevalence of pernicious anemia, a variant of megaloblastic anemia is estimated to be over two million in Pakistan<sup>7,8</sup>.

A possible explanation of folate and vitamin B12 deficiency in older children in our country could be due to various chronic inflammatory disorders of the gut like chronic diarrheas and malabsorptive states apart from poor nutrition<sup>3</sup>. In adults, B12 deficiency is the cause for MGA. In addition, certain anti-tumor or immunosuppressive drugs may also cause MGA<sup>1</sup>.

### Role of vitamin deficiency and folic acid in erythropoiesis:

Cobalamin (B12) and folic acid are required in erythropoiesis<sup>9</sup>. Cobalamin is an essential co-factor for two enzymes, Methylmalonyl Co-A mutase and Methionine synthase. The later enzyme

helps in DNA synthesis by converting methyl tetrahydrofolate into tetrahydrofolate (THF). The deficiency of this vitamin leads to impaired proliferation and morphological changes in all dividing cells. The obvious changes observed in the hemopoietic cells includes megaloblastic erythropoiesis, giant metamyelocytes in the marrow, increase number of hypersegmented neutrophils, macrocytes and elevated mean corpuscular volume in the peripheral blood<sup>10</sup>. Vitamin B12 is also essential for the cells of central nervous system<sup>9</sup>. Its deficiency may also produce various neurological manifestations i.e., parasthesia, numbness, ataxia and psychiatric disorder<sup>11</sup>.

Folic acid, on the other hand is essential for the synthesis of thymine. When thymine is diminished, DNA synthesis proceeds without it by substituting another more available pyrimidine base, uracil, in its place in a futile process called "exision repair". But the uracil misincorporated DNA strands are not the same, and exhibit nicks and breaks. Thus the morphology of folate deficient cells is somewhat of bizarre shapes and sizes, with stretched, oversized cytoplasm and nuclei<sup>12</sup>. It has been documented that when these deficiencies are severe, they affect all the rapidly growing (DNA-synthesizing) tissues giving them megaloblastic changes because DNA synthesis is diminished<sup>13,14</sup>.

#### Cellular changes in megaloblastic anemia

Laboratory studies suggest that megaloblasts are erythroid precursor with enlarged nucleus containing coarse motley chromatin clumps, providing a check-board appearance and show ineffective erythropoiesis<sup>13, 15</sup>. Hematological findings in macrocytic anemia are oval macrocytosis, hyper segmented granulocytes, and anisopoikilocytosis<sup>13,15</sup>.

A similar picture can be seen affecting the epithelial cell surfaces of the mouth, stomach, small intestine, respiratory, urinary and female genital tracts. The cells show macrocytosis, with increased number of multinucleate and dying cells<sup>13,16,17</sup>. Rubin and Massey<sup>18</sup> briefly reported the findings of enlarged nuclei in gastric mucosal cells from gastric washings of patients with pernicious anemia. Graham and Rheault<sup>19</sup> then described enlargement of both nucleus and cytoplasm of squamous

epithelial cells in gastric washings (cells presumably derived from swallowed saliva). A similar abnormality had been seen in a vaginal smear, but no data were given.

The results showed that similar changes are indeed found in saliva of patients with smooth tongues due to iron-deficiency and other causes, although not to the same degree as in certain cases of megaloblastic anemia<sup>20</sup>.

These changes though have not been fully understood in the buccal mucosa in these patients, but account for the phenomena such as glossitis which may be the presenting sign of folate or vitamin B12 deficiency<sup>4, 13, 21</sup>. Several studies have been conducted that reveal the direct effect of folate or vitamin B12 deficiency on the cells of buccal mucosa and gave reproducible results<sup>21, 22, 23</sup>.

#### Diagnostic evaluation of megaloblastic anemia

Nonetheless, adverse effects of anemia indicate that it is an area of public health that needs attention. Diagnosing this disease assumes great clinical importance since it responds exceedingly well to treatment with vitamin supplements. Different diagnostic tests are ordered to identify megaloblastic anemia<sup>15</sup>.

Peripheral blood findings show macrocytosis with or without anemia. The total white cell and platelet counts may be reduced. Neutrophils may show hyper segmented nuclei as an early sign of megaloblastosis and persist for many days after treatment. Reticulocyte count is low. Mean cell volume (MCV) is usually more than 96 fl. Basophil stripling may also be seen.

Bone marrow examination offers detailed information about the condition of the hematopoietic cells. Bone marrow is essentially a blood factory that is normally rich in young cells. Examining bone marrow gives a detailed picture of the quality and quantity of these newly forming blood cells<sup>24</sup>. It is hypercellular with increase in erythroid precursors and there is reversal of myeloid erythroid ratio. Erythroblasts exhibit nuclear and cytoplasmic asynchronization. The granulocyte precursors show giant metamyelocytes and myelocytes.

Unfortunately, Vitamin B12 or folate level fluctuates and administration of any of these

vitamins, which the patient might have received only once, the levels become very high. For these reasons the cause of anemia may become obscure and a bone marrow examination can be the only true evidence of megaloblastic anemia. However, the bone marrow examination is a painful invasive procedure. Unfortunately, the above screening and diagnostic methods have their own limitations and specifications. These may show variations; therefore further work-up is still required.

In view of the voluminous literature and studies already mentioned, reporting the epithelial manifestations of MGA, it has been found that the oral cavity might well be thought of as the window to the body as many oral manifestations are first to happen accompanying systemic diseases<sup>25</sup>, which can be used in the diagnosis of this disease.

## DISCUSSION

The earliest cytological abnormalities to be recognized in MGA were, of course, those of the blood. However, sometimes, peripheral blood findings may not offer enough detail and further evaluation with invasive procedures like bone marrow examination becomes the investigation of choice.

More recently, the granular leukocytes were found to be hyper segmented, and after the introduction of marrow puncture the gigantism of the immature granular cells became an equally familiar feature of this group of diseases. However, bone marrow examination poses risks which include excessive bleeding particularly in people with a low platelet count or people taking aspirin or anticoagulants, such as warfarin; infections, especially in people with compromised immune systems; breaking of needle within the bone, which may cause persistent infection or bleeding; penetration of the breastbone (sternum) during sternal aspirations, which can cause heart or lung problems; long-lasting pain and complications related to intravenous sedation, such as an allergic reaction, nausea or irregular heartbeats<sup>26</sup>.

Historically, Miller<sup>27</sup> first studied exfoliated epithelial cells in human saliva in 1890. It has only been during the past two decades that oral exfoliative cytology has received considerable

attention. The papanicolaou technique and its modification have also been used to study many pre-malignant and malignant lesions<sup>28, 29, 30</sup>, to assess irradiation effects on malignant oral neoplasms and normal tissues<sup>31</sup> and the cytological changes of oral mucosal cells in smokers<sup>32</sup>; alcoholism<sup>33</sup>; tobacco, beetle quid chewers<sup>34</sup> and diabetics<sup>35</sup> that revealed distinguishable results.

Many studies<sup>24, 36, 37</sup> confirm that the buccal cells in pernicious anemia exhibit nuclear enlargement. However, these changes were observed without relating the changes to the state of the oral mucosa, hence it may be suggested that the specificity of the abnormality remains unproved. On the other hand, however, one study found out that the nucleus was reduced in size in all those cases who underwent treatment<sup>20</sup>. Thus, it appears that, in vitamin-B12 deficiency and in the other anemias which respond to folic acid, obvious abnormalities of the buccal squamous cells are the rule rather than the exception<sup>24</sup>.

These results associated with clinical observations suggested that MGA can produce alterations in oral epithelial cells, detectable by microscopy and cytomorphometry, which can be used in the diagnosis of this disease.

## CONCLUSION

The cellular findings associated with MGA have been limited to a very few studies and offers a good research scope for more studies to be conducted<sup>38,39,40</sup>, therefore, it has been suggested that, healthcare strategies that consider the impact of laboratory tests on the overall costs and quality of care should consider the advantages of including cytological findings of buccal mucosal scrapings for evaluation of these patients and can serve as a prerequisite of diagnosing this disease and taking all the literature into account, cytologic findings of buccal scrapings can be a feasible diagnostic and non-invasive technique to identify this type of anemia<sup>41</sup>.

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