ORIGINAL ARTICLE

Haematological Parameters and Recurrent Aphthous Stomatitis

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ABSTRACT

Objective: To find out the relationship between recurrent aphthous stomatitis (RAS) with deficiencies of haemoglobin, haematocrit, serum vitamin B12, serum Ferritin and red blood cells (RBC) Folate level.

Study Design: An analytical cross-sectional study.

Place and Duration of Study: Department of Oral Health Sciences, Shaikh Zayed Federal Postgraduate Medical Complex, Lahore, from February to July 2008.

Methodology: Sixty consecutive subjects with active RAS were taken as the aphthous group; 60 age and gender matched subjects without RAS were as the Non-Aphthous group. Five milliliter blood was taken from both groups to evaluate the levels of serum B12, and RBC Folate through radio immuno assay and serum ferritin with enzyme linked immuno-sorbent assay tests. Complete blood count was carried out to determine the level of haemoglobin and haematocrit in both groups. Proportion of subjects with lower values was compared using 2 text of proportions with significance at $p < 0.05$.

Results: Serum Ferritin ($p = 0.001$), haematocrit ($p < 0.001$), RBC Folate ($p < 0.001$) and serum B12 ($p < 0.001$) were significantly lower in the RAS group. Combined deficiency state (haemoglobin, serum Ferritin, haematocrit, RBC Folate and serum B12) was identified in 13% ($n = 8$) RAS patients.

Conclusion: Frequency of haematinic deficiencies was high in RAS patients. Serum B12 and RBC Folate were significantly low in aphthous group.

Key words: *Recurrent aphthous stomatitis. Deficient haemoglobin. Haematocrit. Serum ferritin. Serum B12. RBC folate.*

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a chronic relapsing, remitting oral mucosal disease, which persists for a variable period of time and affects nearly 50% of the world population.[1,2] Immunological factors, local trauma, genetic and microbial factors and haematinic deficiencies are the major predisposing factors.[1,2]

Haematinic deficiencies (B12, folate, and iron) cause nutritional anaemia and is common among infants, young children, menstruating and pregnant women in developing countries.[3] RAS when uncomplicated, may be associated with haematinic deficiency since the latter causes atrophy of oral epithelium.[4] Thinning of the epithelium makes it more venerable to trauma and increases penetration of exogenous bacterial antigens, one of the factors implicated in RAS.[2]

Several studies have suggested the importance of iron, folic acid and vitamin B12 deficiencies and nutritional intolerance; however, some controversies exist. Carrozzo found no statistically significant difference between the haematological parameters in RAS patients and control group,[5] whereas Porter reported complete elimination of RAS after haematinic replacement therapy.[6] Recent preliminary and controlled studies observed reduced or eliminated recurrences of RAS lesion and 74.1% RAS patients recovered when prescribed haematinic replacement therapy.[7] In Pakistan two and a half million people are nutritionally anaemic out of whom 11% were suffering from pernicious anaemia which causes oral ulceration, mucosal bleeding and glossitis.[8]

Although RAS is a public health problem and nutritional anaemia is also prevalent in Pakistan, no work has been done to investigate any correlation between RAS and haematinic deficiencies. Very limited data is available regarding RAS which mainly described its correlation with intestinal parasitosis.[9] For this study, determination of association between RAS and vitamin B12, serum Ferritin, RBC Folate, haemoglobin, and haematocrit deficiency was the objective.

METHODOLOGY

This study was carried out at Outpatient Department (OPD) of Oral Health Sciences, Shaikh Zayed Federal Postgraduate Medical Complex, Lahore, from February to July 2008. Permission to carry out this study was taken from the Ethical Review Committee Ref. No. SZMC/IRB/536/338 and Institutional Review Board (IRB)-Number 1054, Shaikh Zayed Postgraduate Medical Institute, Lahore. It was a non-probability sampling technique. Study population comprised of two groups including Aphthous Group (AG) and Non-Aphthous Group (Non-AG).

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Sixty patients attending dental OPD who had active RAS during the last 6 months were selected as Aphthous Group. Patients with systemic diseases including Behcet's disease, Crohn's disease, ulcerative colitis, uremic stomatitis,[10] subjects taking RAS treatment or sulphonamides, rifampicin and vincomycin, cytotoxic agents like methotrexate and non-steroidal anti-inflammatory drugs[11] were excluded from the study. The other group without ulcers during last 6 months was selected as Non-Aphthous group or control group.

All the participants agreed to participate in the study after taking written informed consent, giving a response rate of 100%. After recording patients' identity, age, gender, occupation, address and history of systemic disorders, 5 ml blood was drawn from AG and Non-AG subjects. Two milliliter was added to EDTA tubes for complete blood count, and analysis was carried out on Sismax machine. Three mililiter was centrifuged at 3000 rpm for 10 minutes for the analysis of serum Ferritin by Enzyme Linked Immunosorbent Assay (ELISA) method and serum B12 and RBC Folate by radio immunoassay procedure.

The data was recorded in a specially designed proforma, which was coded and entered in Statistical Package for Social Sciences (SPSS) version 15.0 for analysis. Demographic variable that is age is presented as mean ± SD and gender is described using frequency and percentage. The levels of haemoglobin, serum B12, serum Ferritin, RBC Folate and haematocrit for both groups are presented in proportions.

The above mentioned data was recoded in to two groups as follows: Haemoglobin was recoded as low and normal using cut-off value of 12 gm/dl. for females and 14 gm/dl for males. Haematocrit (Hct) cut-off value was taken 36% between low and normal. Serum B12 level cut-off was taken as 220 pg/ml. Serum Ferritin cut-off value for females was taken ≤ 10 ng/ml, and for males at ≤ 20 ng/ml. Serum Folate cut-off value for low and normal was taken at 1.5 ng/ml.

Recoded values were compared by using Z-test for proportions. For all analyses, a p-value < 0.05 was considered significant.

RESULTS

The AG had 38 females (63.3%) and 22 males (36.6%) and Non-AG had 33 females (55.0%) and 27 males (45.0%). Age ranged from 8 to 55 years and the mean age was 27.1 ± 11.14 years. Twelve male and 22 female subjects in AG group were less than 30 years of age while 10 males and 12 females were above 30 years. In Non-AG group 17 males and 25 females were below thirty and 10 males and 8 females were above thirty.

Minor RAS cases with ulcer size 8 – 10 mm in diameter were seen in 85% (n = 51) of the subjects, 11.7% (n = 7) patients suffered major RAS (> 1 cm in size), and two subjects (3.3%) had Herpetiform RAS with ulcer size less than 8 mm.

Table I: Comparison of haematological parameters between aphthous group and non-aphthous group.

Haematological parameters	AG (n = 60)	Non-Ag (n = 60)	p-value*
Low haemoglobin (all)	35 (58.3%)	26 (43.3%)	p = 0.096
Male (22, 27)	21 (95.5%)	26 (96.3%)	p = 0.825
Female (38, 33)	14 (36.8%)	0 (0.0%)	p < 0.001
Cut off value female ≤ 12 g, male's ≤ 14 g			
Low haematocrit			
Cutoff value 36%	20 (33.30%)	5 (8.0%)	p < 0.001
Low serum B12			
Cut-off value ≤ 220 pg/ml	27 (45.0%)	9 (15.0%)	p < 0.001
Low S. Ferritin	19 (31.7%)	7 (12.0%)	p = 0.007
Male (22, 27)	8 (36.4%)	4 (15.0%)	p = 0.005
Female (38, 33)	11 (28.9%)	3 (9.0%)	p = 0.004
Cut off value ≤ 10 ng/ml FM, ≤ 20 ng/ml M			
Low Folate			
Cut-off value < 1.5 ng/ml	31 (51.7%)	6 (10.0%)	p < 0.001

**Analysis based on Z-test for proportions.*

There were 35 subjects (58.3%) with low Hb level in AG while 26 (43.3%) in Non-AG (p = 0.096). In males there were 21 subjects (95.5%) with low Hb level in AG while 26 (96.3%) in Non-AG (p = 0.817). In females, there were 14 subjects (36.8%) with low Hb level in AG while 0 (0.0%) in Non-AG group (p < 0.001, Table I).

There were 19 subjects (31.7%) with low Ferritin level in AG while 7 (12.0%) in Non-AG (p = 0.007) group. While in males there were 8 subjects (36.4%) with low Ferritin level in AG while 4 (15.0%) in Non-AG (p = 0.005) group. In females, there were 11 subjects (28.9%) with low Ferritin level in AG while 3 (9.0%) in Non-AG (p = 0.004, Table I).

Subjects in the AG group having low level of Hct were 20 in number (33.3%). In comparison, subjects in Non-AG group having low level of Hct were 5 (8.0%, p < 0.001, Table I).

Twenty seven (45.0%) subjects of AG group were deficient in B12. In comparison, 9 (15.0%) Non-AG subjects were deficient in B12 (p < 0.001, Table I).

Subjects belonging to AG having low serum Folate were 31 in number (51.7%). In comparison, Non-AG subjects with low serum Folate value were 6 (10.0%, p < 0.001, Table I).

DISCUSSION

The study was conducted to evaluate the relationship between haematinic deficiencies and RAS. The AG subjects included individuals with a mean age of 27.1 ± 11.14 years. Separate Non-AG with age and gender matched individuals was selected for comparison.

According to published studies, 80% of all RAS lesions are minor RAS in nature.[1] This was witnessed in the

current study as well where 85% (n = 51) cases were minor RAS in nature. Results of current study found 65% patients (38/60) of AG belonged to age group of 16 – 30 years (n = 23) and the rest (n = 15) were 31 – 44 years. The demographics of this study are consistent with previous studies,[11] as there were more females than males in the AG group. This may be explained by the fact that adult women frequently encounter aphthae or histories of aphthae because they have high predisposition to become anaemic.[3]

In the present study, there was low level of haemoglobin (< 11.5 g/dl) both in AG and Non-AG with 42% of the total sample (n = 120). RAS patients more frequently had deficient Hb level as compared to controls.

Helay observed anaemia in 10.5% RAS patients.[2] Some studies found the level of Hb within normal range both in study and control group.[13] This difference in anaemia level can be attributed to the fact that 49% of females and 19% of males of the total Pakistani population are anaemic and also anaemia is not distributed uniformly among adults in Pakistan.[14]

Deficiency of iron is one of the causes of anaemia. In Pakistan, iron deficiency anaemia based upon serum Ferritin level was observed to be moderate anaemia in 53% and severe anaemia in 47%.[15] Many studies have reported Ferritin deficiency in RAS patients[2,16] with varying frequencies ranging from 96.5%[2] to 37%.[16] Present study supports these findings, as 31.7% RAS patients had low Ferritin level as compared with controls 12.0%. Levels of Ferritin were found to be low in the current study, which can be explained by the fact that anaemia is common in developing countries including Pakistan.

Haematocrit (Hct) deficiency can be seen in a variety of conditions (blood loss, malignancy, nutritional deficiency).[17] The level of haematocrit was markedly low in both AG and Non-AG subjects (33.1%) in this study. Thirty three percent RAS patients (n = 20) had low Hct as compared to controls (8.0%, n = 5), supported by Helay who found low Hct in 31.5% RAS patients.[2] Reason of low haematocrit was probably a low iron intake, or heavy blood loss during menstruation and delivery. Other researchers did not investigate the levels of Hct.[6]

Recurrent aphthous stomatitis when uncomplicated, may be associated with deficiency of Folic acid and serum B12. Serum B12 is demonstrated to be the most frequently found haematinic deficiency; its deficiency suppresses the cell mediated immunity,[18] and changes in the epithelium of tongue and the buccal mucosa.[19] Serum B12 is essential for the synthesis of DNA, and its deficiency results in the development of megaloblastic anaemia in developing countries including India[20] and Israel.[21]

In developed countries like USA,[12] and Denmark,[16] the frequency of serum B12 deficiency is 1 – 6% in adults and children[6] while in teenagers and adults having RAS lesions, the frequency of serum B12 deficiency was 23%.[22] In India 36.9% males and 46% females had low B12,[20] while in Pakistan the deficiency of B12 was reported to be upto 72% (n = 50).[23]

Serum B12 deficiency was found to be 33.3% in the total study population (n = 120) in current study. Serum B12 was low in 45.0% (27/60) of RAS patients and 15.0% (9/60) of controls ($p < 0.001$). Low level of serum B12 in Pakistan is due to low intake of meat, as it is not available to majority of the population due to poverty, and may also be due to the lack of awareness of balanced diet.

Studies conducted by Burgan observed 26.6% deficiency of B12 in RAS patients.[16] Majority of RAS patients are deficient in serum B12.[22,24] Cobalamine injection makes 74.1% of these subjects free from aphthous ulcers at the end of the treatment.[22]

Folic acid enhances the regeneration and healing of oral epithelium in response to damage. It is also essential for the synthesis of DNA.[25] Deficiency of Folate in adult life produces megaloblastic anaemia.[23] The level of Folate deficiency in Pakistan is 16%.[23]

Current study showed statistically significant difference between RBC Folate levels between AG and Non-AG. These results correspond with that of Barnadas.[26] Subjects of poor vegetarian families are more prone to Folate deficiency. Majority of our population is of low income group and proteins are not easily assessable to these subjects. Infants and young children who are not weaned adequately are particularly susceptible.

There is a need to carry out interventional studies to find out the causative relationship between RAS and haematological parameters. That may help perform a valid analysis and suggest a definitive role of nutritional deficiencies in the occurrence of recurrent aphthous stomatitis.

CONCLUSION

Frequency of haematinic deficiencies was high in RAS patients. Serum Ferritin, serum B12, and RBC Folate deficiencies were significantly greater in RAS patients as compared to controls.

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