Original Article

# Evaluation Of Hematological Parameters Of Patients With Recurrent Apthous Stomatitis Among Sriganganagar Population – A Case Control Study

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## Abstract

Introduction: Recurrent Apthous Stomatistis (RAS) is defined as the presence of recurring ulcers confined to oral mucosa. Approximately 20% of patients suffer from this painful and debilitating disease. Present study was conducted to assess the level of Serum B12, Serum Feritin, Hemoglobin and Foliate as the risk factor of recurrent apthous stomatitiss and to know whether blood grouping has any influence in Recurrent Apthous Stomatitis.

Aim: The aim of the study was to investigate various haematological changes in patients with common oral ulcers.

Method**:** 415 people were included in the study, 212 cases and 203 control subjects, consisting of 126 men and 289 women (age range: 18-70 years). Patients were diagnosed as having RAS after they had at least three episodes of oral ulcerations. The variable assessed were Serum Feritin, Serum vitamin B12, Haemoglobin and Serum iron, folic acid, and blood groups. Data collection was done using a questionnaire with their demographic details, frequency and healing period of ulcers. Using WHO guidelines, diagnostic criteria was setup. Blood samples were collected comparisons were done. Data was analyzed,Chi-squaree and Mann Whitney U test was applied to test significant differences between cases and controls, p<0.05 was considered statistically significant.

Results: Majority of lesions were minor type, followed by RAU major and herpetiform ulcers. Overall, hematological parameters in the study group were lesser than the control group, which was statistically significant. It was observed that RAU patients majorly were of blood group O, followed by B, A and AB.

Conclusion: Hematological disturbances may be an etiological factor of RAS. Greater emphasis must be placed on balanced nutrition to RAS patients.

**Keywords:** Aphthous Stomatitis, , vitamin B12, Serum iron, folic acid, Blood group

## INTRODUCTION

The term aphthae is derived from the Greek word aphthi, which means “to set on fire “or “to inflame”. it was first described by the Great philosopher Hippocrates as pain associated with a common disorder of oral mucosa during his time (Recurrent Aphthous Stomatitis).[1]Recurrent aphthous stomatitis (RAS) is defined as the presence of recurring ulcers confined to the oral mucosa in patients with no other signs or symptoms of underlying disease. The disease presents in three classic forms namely minor, major and Herpetiform types.[2,3]

There are numerous studies done for knowing the exact etiology of RAS, among them the abnormality in Host factors such as Genetic, Immunological, Hematological, Vitamin deficiency, Physical or Emotional stress, and environmental factors such as microtrauma, chemical injury and infections have been implicated. Hematinic deficiency (Serum Ferritin and Serum Vitamin B12) in particular was found to be twice as common.[4]

Its prevalence varies among different socioeconomic groups and its incidence is strongly affected by family history[5]. RAS is the most common type of recurrent oral ulceration in the age group of 10 to 19 years. And also, similar ulcers can be seen in patients with a variety of diseases including systemic lupus erythematosus, Bechet’s syndrome, iron deficiency

anemia and Crohn's disease. Therefore, RAS patients must be carefully evaluated for associated symptoms to determine the underlying pathology and whether laboratory evaluation is required [6].

## MATERIALS AND METHODS:

A total of 415 people were included in the study, 212 (51.1%) cases and 203 (48.9%) control subjects, consisting of 126 men and 289 women (age range: 18–70 years). Various parameters of laboratory investigation were assessed in terms of Serum Ferritin Serum vitamin B12, Hemoglobin (Hb), Serum iron, folic acid and Blood group in the patient and control group. Patient demographic information was recorded in case history Performa and patients were subjected to thorough clinical examination under artificial illumination with the help of a mouth mirror for the diagnosis of recurrent aphthous stomatitis based on criteria proposed by Natah et al in 2004 and data was recorded by the questionnaire provided to them regarding the frequency and healing period of ulcers which occurred. And by examining the lesions, as round symmetrical yellowish white ulcers, less than 1 cm in diameter with an erythematous halo diagnosis was made by oral medicine specialist. For the haematological investigation, blood samples were collected from each participant after an overnight fast. The blood group of the cases and controls was collected by a single trained and calibrated investigator using the finger prick method and blood typing done by tube method. After that patients were classified according to blood group (A, B, AB and O). About 3ml blood was drawn from cases and controls which was centrifuged at 3000 rpm for 10 minutes for the analysis of serum Ferritin by Enzyme Linked Immunosorbent Assay (ELISA) method and serum B12 by radio immunoassay. The clinical laboratory responsible for the blood tests was also blinded to the group allocation. Serum vitamin B12 levels were measured using an Access Vitamin B12 Assay Kit (Beckman Coulter, Brea, CA). The accepted normal serum vitamin B12 level is 180–914 ng/L. Serum ferritin were measured using an Access Ferritin Assay Kit (Beckman Coulter, Brea, CA). The accepted normal serum ferritin level for females is 11.0–306.8 ng/ml, and for males is 15–336.2 ng/ml. Serum folate levels were measured using an Access Folate Assay Kit (Beckman Coulter, Brea, CA). The accepted normal serum folate level is 4.0–18.7 ng/ ml. Serum vitamin B12, ferritin, and folate deficiencies were defined as serum levels below their cut-off values in all cases.

## STATISTICAL ANALYSIS

The results obtained in this case-control study was undertaken to evaluate haematological parameters in patients affected with Recurrent Aphthous Ulcers. Data was analyzed, Chi square and Mann Whitney U test was applied to test significant differences between cases and controls, p<0.05 was considered statistically significant. Data analysis was done by using SPSS 23.0 version. Majority of the lesions were minor type, followed by RAU major and herpetiform ulcers. Overall, hematological parameters in the study group were lesser than the control group, which was statistically highly significant. It was observed that RAU patients majorly were of blood group O, followed by B, A and AB.

## RESULTS

A clear female predilection was noted with 289 (69.6%) of the study participants being females. 127 females were affected with the condition as against 85 males which was highly significant at p<0.001. (Table 1)

**Table 1:** Gender distribution across the groups

| Gender | Recurrent Apthous Ulcers | | Total N (%) |
|---|---|---|---|
| | Present N (%) | Absent N (%) | |
| Males | 85 (20.5) | 41 (9.9) | 126 (30.4) |
| Females | 127 (30.6) | 160 (39.0) | 289 (69.6) |
| Total | 212 (51.1) | 203 (48.9) | 415 (100) |

Chi-square test – 19.418; df – 1; p <0.001** (Highly Significant)

**Table 2:** Age distribution in study population

| Groups | N | Mean ± S.D |
|---|---|---|
| Study | 212 | 38.400 ± 3.729 |
| Control | 203 | 40.200 ± 7.592 |
| Total | 415 | 39.300 ± 6.041 |

Mann Whitney U test – 1920.0; p = 0.487 (Not Significant)

The mean age of study participants were 39.300 ± 6.041. There was no significant difference between the ages of study group (38.400 + 3.729) and control group (40.200 + 7.592) (Table 2)

**Figure 1:** Age distribution of study participants

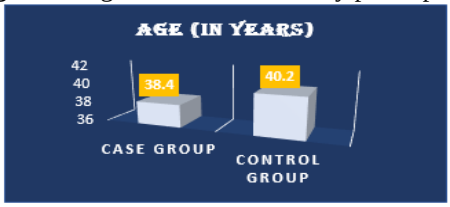


**Table 3:** Type of Lesions in Study group

| Lesion type | N | % |
|---|---|---|
| Minor | 138 | 65.09 |
| Major | 49 | 23.11 |
| Herpetiform | 25 | 11.79 |
| Total | 212 | 100.00 |

Majority of the lesions were of minor type, followed by RAU major and herpetiform ulcers. (Table 3)

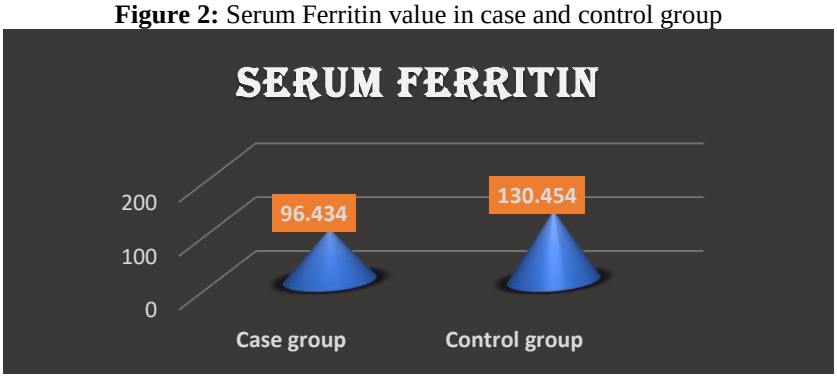
**Table 4:** Haematological variables in study population

| Groups | Ferritin (ng / ml) | Haematocrit (%) | Vitamin B12 | Folic acid | Haemoglobin |
|---|---|---|---|---|---|
| Study | 96.434 ± 6.913 | 37.800 ± 2.863 | 593.000 ± 277.825 | 7.740 ± 1.223 | 12.592 ± 1.984 |
| Control | 130.454 ± 11.233 | 48.400 ± 2.734 | 647.400 ± 237.7728 | 10.822 ± 1.816 | 14.642 ± 1.147 |
| Total | 113.440 ± 19.412 | 43.100 ± 5.998 | 620.200 ± 259.663 | 9.281 ± 2.184 | 13.617 ± 1.917 |
| Mann Whitney U test | 2100.00 | 3920.00 | 1600.00 | 4800.00 | 9600.00 |
| P value | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** |

** = Highly Significant; * = Significant; NS = Nothing Significant

Overall, haematological parameters in the Study group was lesser than the control group, which was statistically highly significant at p<0.001. (Table 4)

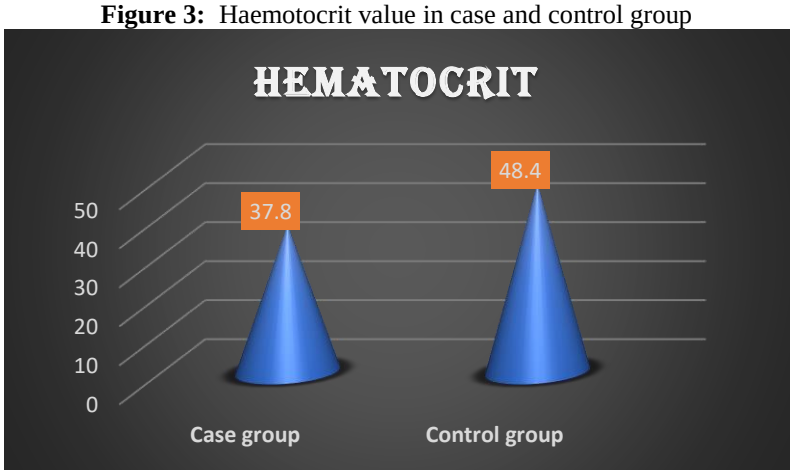
**Figure 2:** Serum Ferritin value in case and control group




Serum Ferritin had a mean value of 96.434 ± 6.913 ng/ml in the case group. The control group on the other hand had scores of 130.454 ± 11.233 ng/ml. (Table 4 and Figure 2)

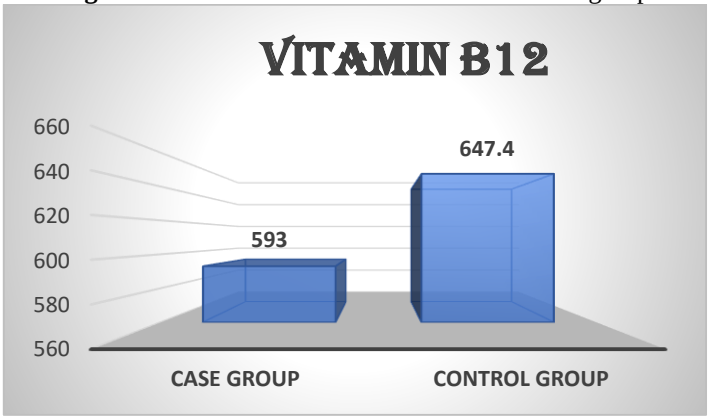
The haematocrit value was reported to be 37.800 ± 2.863 in the population with RAU while it was 48.400 ± 2.734 in the control group. (Table 4 and Figure 3)

**Figure 3:** Haemotocrit value in case and control group



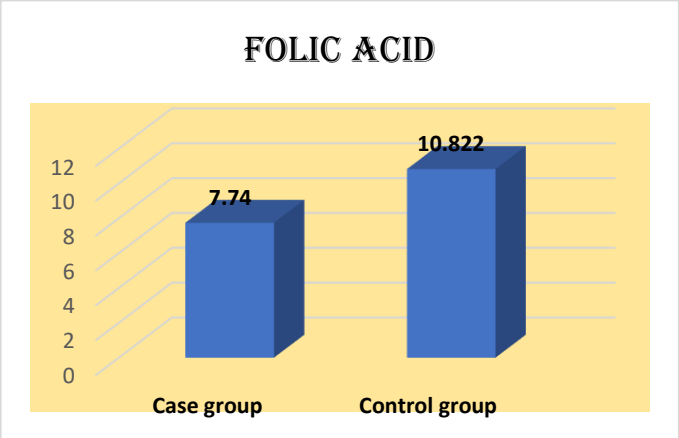

Vitamin B12 had a mean value of 593.000 ± 277.825 in the study group and was much higher in the control group (Table 4 and Figure 4)

**Figure 4:** Vitamin B12 value in case and control group




Folic acid levels were lesser in the RAU group with a mean of 7.740 ± 1.223 and it increased to 10.822 ± 1.816 in the control group. (Table 4 and Figure 5)

**Figure 5:** Folic acid value in study population




Haemoglobin values were higher in the control group than the study group (14.642 ± 1.147 versus 12.592 ± 1.984) (Table 4 and Figure 6)

**Figure 6:** Haemoglobin value in study population

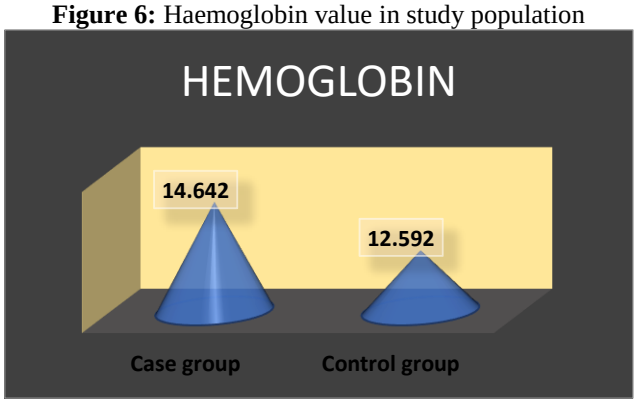



**Table 5:** Blood group distribution among cases and controls

| Blood groups | Cases N (%) | Controls N (%) | Total N (%) |
|---|---|---|---|
| Blood group A | 43 (10.4) | 26 (6.3) | 69 (16.6) |
| Blood group B | 55 (13.3) | 55 (13.3) | 110 (26.5) |
| Blood group AB | 13 (3.1) | 81 (19.5) | 94 (22.7) |
| Blood group O | 101 (24.3) | 41 (9.9) | 142 (34.2) |
| Total | 212 (51.1) | 203 (48.9) | 415 (100) |

Chi square test – 78.574; df – 3; p <0.001** (Highly significant)

In the blood grouping, it was observed that RAU patients majorly were of Blood group O, followed by B, A and AB. In the control group, majority of the patients were of AB blood group, which was statistically significant at p<0.001. (Table 5)

## DISCUSSION

Oral health is closely linked to general health, with the oral cavity often revealing early signs and symptoms of certain underlying systemic diseases. Thus the present study was done to evaluate various hematological parameters in patients affected with Recurrent Aphthous Ulcers versus the control population. Total 212 cases and 203 controls were evaluated for the present study.

Recurrent aphthous stomatitis (RAS) is a chronic inflammatory relapsing, remitting oral mucosal disease, which continues for a variable period of time and affects virtually 50% of the world population, with three classic forms namely minor, major and Herpetiform types.[7,8] In this study, females were more frequently affected than males results were similar when compared to the findings of Santosh et al (56.3% females and 43.7% males) and Rajmane YR (62.5% females and 37.5% males). This might be attributed to more stress and hormonal changes in females as compared to male [9,10]

The etiopathogenesis of hematinic deficiencies causing RAS is still unclear, however, outbreaks from oral epithelial atrophy, injury to mucous membrane barrier, and negative influence on the epithelial integrity were reported as the primary mechanisms behind it. Hematinic deficiency (Serum Ferritin and Vitamin B12) in particular was found to be twice as common [11] The present study showed lower levels of serum Ferritin with a mean value of 96.434 $\pm$ 6.913 ng/ml in the case group while the control group on the other hand had scores of 130.454 $\pm$ 11.233 ng/ml. Alberto Rodriguez-Archilla et al showed similar results in their systematic review with RAS patients to have 6.67 times more likely to have low iron levels, with highly significant statistical differences (OR = 6.67, 95% CI: 2.37–18.77, P < 0.001). The patients of 11 studies with RAS had significantly lower iron concentrations (11.75 µg/dL lower on average) compared to controls (P < 0.001). Tidgundi et al also reported lesser iron levels in his RAS group. Iron deficiency is responsible for anemia in which the decrease in the number of erythrocytes results in less oxygen transport to tissues, including the oral mucosa. This lack of oxygen affects the differentiation and maturation of the epithelial cells causing the atrophy of the oral mucosa. This atrophy increases tissue susceptibility favoring antigenic exposure and the development of a cytotoxic immunological response that could lead to RAS [12,13].

**Vitamin B12** had a mean value of 593.000 $\pm$ 277.825 in the study group and was much higher in the control group in our current study. Bao ZXet also reported similar results with B12 deficiency in RAS groups[14] Ghafoor F also reported lesser B12 in patients with RAS (124.12±46.5) as compared to controls (258.91±111.6) (47). Vitamin B12 deficiency, either separately or in combination with other deficiencies of the Vitamin B complex, can be a possible precipitating factor for RAS. Vitamin B12 deficiency suppresses the cell-mediated immunity, changes in the epithelium of tongue and the buccal mucosa.[15,16] Serum B12 is essential for the synthesis of DNA, and its deficiency results in the development of mega loblastic anaemia, especially in developing countries including India.[17] Vitamin B12 deficiency impairs the metabolism of folate which can lead to folic acid deficiency. Therefore, in addition to Vitamin B12 measurement, folic acid should also be measured in cases of RAU.

The current study demonstrated lesser amounts of folic acids in RAS cases than in controls. The possible relationship between folic acid and RAS is also investigated in literature. Rodriguez-Archilla A investigated folic acid concentrations in their systematic review with RAS and in controls. Patients showed lower folic acid concentrations (0.95 ng/mL lower on average) compared to controls with very significant statistical differences (P = 0.001).

Blood group antigens are not only expressed on red blood cells but also expressed in epithelial cells and they have been known to play a role in several biological processes including bacterial adhesion, inflammation, cell movement and tissue differentiation.[18] Hence blood group antigens, by playing a role in immunity and genetics could also attribute to the etiopathogenesis of RAS. In the blood grouping, it was observed that RAU patients majorly were of Blood group O, followed by B, A and AB. In another similar study that was done among 200 study participants, it was found that the O blood group individuals had a higher prevalence of RAS when compared to other blood groups and the least was found among the AB blood group individuals[19,20].

The only limitation of this study is that it was conducted only for a short period of time about 4 months, the long term value of improvement needs to be confirmed by further longitudinal studies with an increase in sample size. Hence, it is recommended that more genetic and molecular level studies should be carried out regarding apthous stomatitis in association with blood group and Rh factor for a longer duration of time as these things might leave a better impact on the literature.

## CONCLUSION

Recurrent apthous stomatitis is one of the most common diseases affecting oral cavity with multifactorial etiology. Different blood groups and haematological variables may constitute a risk factor on the development of aphthous

stomatitis. Hence we as oral physicians must consider RAS as secondary effect of hematinic deficiencies and treat patients accordingly with greater emphasis focussed on balanced diet with good nutrition supplements to RAS patients.

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