MICRO-ORGANISMS IN RECURRENT APHTHOUS STOMATITIS.

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OBJECTIVE: Recurrent aphthous stomatitis is an inflammatory disease of oral mucosa with unknown etiology. Many factors contribute in the pathogenesis of aphthae including stress, trauma, foods, allergy, hormonal and microbial factors. It has been observed by Hoover CI (1986) that microorganisms are the causative factor in the pathogenesis of aphthous ulcer. This study was conducted with the aim to observe presence or absence of gram positive and gram negative microorganism in the smears of the lesions compared with the scraping of controls.

METHODOLOGY: Study Design: An Analytical cross-sectional study.
Place of Study: It was carried out in Dental Out Patient Department of Shaikh Zayed Federal Postgraduate Medical Institute, Lahore. For a period of Six months First 60 subjects with active RAS during last six months were recruited for the study. Age and gender matched 60 subjects without ulcer during last six months were selected on the same day as control group. After taking informed consent, smears from RAS patients and scrapings from matched surfaces of the oral mucosa of controls was taken.

RESULTS: Gram +ve and Gram ve cocci and bacilli were the microorganisms frequently found in the smears of RAS subjects and Gram +ve cocci were observed in the scrapings of Controls.

CONCLUSION: Presence of microorganisms in ulcers suggested that there is superimposed bacterial infection in these lesions.

KEY WORDS: aphthous ulcers, microorganisms.

INTRODUCTION
Recurrent aphthous stomatitis (RAS) is one of the most commonly seen oral mucosal disorder that affects about 66% of the population depending on the sample evaluated. Clinically RAS can be divided into Minor, Major and Herpetiform types. Minor RAS or MiRAS is less than 1 cm in diameter, and is the commonest form of RAS that affects about 80-85% of all RAS patients. Lesions of MiRAS heal within 10-14 days without scar formation. Major RAS (MjRAS) is a severe form which comprises 5-10% of RAS sufferers. The size is 1cm in diameter, takes weeks to months to heal with scar formation. Herpetiform RAS or HuRAS is the least common form of RAS which affects 5-10% of RAS patients. Although a considerable amount of research was carried out the chapter of its etiology is still open. Predisposing factors causing this painful condition include stress, trauma, drug and food allergy, inflammatory bowel disease, Pemphigus vulgaris, infections and microorganisms. As the etiology is unknown so the treatment is palliative. Recent studies conducted evaluate that RAS lesions are due to the reaction of autoimmunity against oral epithelium. Autoimmune reaction could be reaction of immune response which may be activated by oral bacteria. Microorganisms present in these ulcers can be observed through Gram staining that can help differentiate Gram positive and Gram negative cocci and bacilli in the lesions.

Recurrent aphthous stomatitis is a common...
complaint of worldwide occurrence, including Pakistan. It is a common disorder which can be faced by dentists and general practitioners in their routine work. Currently, no work has been carried out to observe presence or absence of microorganisms to cure this debilitating condition and give proper treatment. This study was therefore carried out to evaluate microorganisms in the ulcer bed for proper management and relief of pain.

METHODOLOGY

It was an analytical cross sectional study carried out in the Dental Out Patient Department of Shakih Zayed Federal Postgraduate Medical Institute, Lahore, Pakistan.

Study Criteria

Sixty subjects with active RAS during the last six months were selected as Aphthous Group. Age and Gender matched Controls were selected on the same day with out ulcer during last six months as Non-Aphthous Group (Non-AG).

Data Collection

Patients name, age, gender, occupation and address were noted. History of any systemic disorder was taken. All the participants agreed to participate in the study after taking informed consent, giving a response rate of 100%. 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, Sheikh Zayed Postgraduate Medical Institute, Lahore. Pakistan.

SAMPLE COLLECTION

Smear collection for microbiological investigation

With the help of sterilized mirror, smear was collected from the site of ulcer from subjects included in Aphthous group and scrapings were obtained from corresponding site of all Non-Aphthous group using the following protocol.

Smears for Aphthous Group

1- Sterilized cotton buds were dipped into sterile saline solution to avoid fiber sticking. Smears were taken gently after wearing sterile gloves and face mask from the lesion of buccal or labial mucosa, tongue, floor of mouth, soft or hard palate or lip and was spread on a clean slide.

2- Air dried smears were fixed by drying with heat and stained by Gram staining method to detect the type of bacteria in the lesion.

Scrapings for Non-Aphthous Group

Specimen for Non-Aphthous Group was taken from the buccal or labial mucosa, tongue, floor of the mouth, or from lip correlating with Aphthous Group mucosal surfaces.

1- Cotton swab was dipped into normal saline.

2- Mucosal scraping was taken from the site comparable with the case. (From tongue, buccal, labial mucosa or floor of the mouth)

3- Slides were prepared and were stained with Gram staining as was done for Aphthous group.

Procedure of Gram staining

The Gram staining reaction was used to identify pathogens in specimens and cultures by their Gram reaction (Gram positive or Gram negative) and morphology (Cocci or Bacilli). Gram positive bacteria stain dark purple with crystal violet and are not decolorized by acetone or ethanol. Staphylococcus, streptococcus, streptococcus, clostridium and corny bacterium belonged to this group. Gram negative bacteria stain red as they are decolorized by acetone after staining with crystal violet. Neisseria, klebsilla, yersinia and hemophilus belong to this group.

1- Dried smear was fixed.

2- The fixed smear was covered with crystal violet stain for 30-60 seconds.

3- The crystal violet stain was rapidly washed off with tap water.

4- Water was tipped off and the smear was covered with Lugol's iodine for 30-60 seconds.

5- Lugol's iodine was washed off with tap water.

6- The smear slides were decolorized rapidly with acetone/alcohol for few a seconds.

7- Finally the smear was covered with neutral red stain for 2 minutes and was then washed with tap water.

8- The back of the slide was wiped to make it clean, and the slide was placed in a draining rack to air-dry the smear.

Microscopic Examination of the slides

After preparation of the slides the smear was examined microscopically first with the 40X objective to check the staining, and to see the distribution of material, and then with oil immersion objective to check bacteria. On Gram staining four groups of bacteria were seen in RAS smears. These were Gram positive and Gram negative Cocci and Gram positive and Gram negative Bacilli.
RESULT

Microbiology of ulcer bed

Presence and Arrangement of Gram Positive cocci in Non-Aphthous group

Gram positive cocci were seen singly and in small clusters in Non-AG.

Presence Of Microorganisms in Aphthous Group:

Gram positive cocci were seen in the smears of 45 samples of Aphthous Group (75%). Forty subjects of Aphthous Group (66.7%) revealed the presence of gram negative cocci in smears of their lesions.

Gram positive bacilli were present in 39 samples (65.0%) of the smears in Aphthous Group. Forty five percent (27 samples) of the smears of Aphthous Group showed presence of Gram negative bacilli.

Gram positive cocci are normal commensals of oral cavity and pharynx. However, their population constantly changes due to significant fluctuations in the relative proportion of existing clones. In our study, scrapings of Non-Aphthous Group showed Gram positive cocci which were normal commensals of oral mucosa with Singles and small clusters.

In this study it was found that there was bacterial diversity of microorganisms in the ulcer group as compared to non-ulcer group (fig 1) as was also observed by Marchini et al in 2007.

Seventy five percent (45/60) of the lesions of RAS showed presence of gram positive cocci. Sixty six percent (40/60) subjects had gram negative cocci in their smears, Gram positive bacilli were present in 64.9% (39/40) samples of RAS subjects and 45% (27/60) subjects had gram negative bacilli in their ulcerative smears. The high load of microorganism in the smears of ulcer bed was also observed in the study of Hasan in 2002.

Humoral and cellular immunity is changed due to streptococcus oralis in RAS patients. Serum antibodies are present in RAS and control group but the mean end-point titres are shown to be significantly higher in RAS patients than controls. In RAS biopsies alpha-hemolytic streptococci, coagulase negative staphylococci and neisseria are dominant organisms.

With a very simple and low cost procedure of Gram staining as done in the present study a suitable topical antibiotic can be prescribed for palliative treatment of aphthous ulcers.

To subside superimposed bacterial infection in RAS.

Table No 1. Microorganism present in the ulcer bed

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Positive Cocci</td>
<td>45</td>
</tr>
<tr>
<td>Gram Negative Cocci</td>
<td>40</td>
</tr>
<tr>
<td>Gram Positive Bacilli</td>
<td>39</td>
</tr>
<tr>
<td>Gram Negative Bacilli</td>
<td>27</td>
</tr>
</tbody>
</table>

DISCUSSION

The current study was conducted to evaluate the presence of microorganisms present in the lesions of aphthous stomatitis. The Aphthous Group or AG (clinically healthy) included individuals of all age groups (8-55 years) with a mean 27.1 years ±SD 11.14. Separate non-aphthous group (Non-AG) with age and gender matched individuals were selected for comparison.
antibiotic mouth wash should be used as previous investigations have shown good response by using tetracycline and Chlorhexidine therapy.

LIMITATION OF THE STUDY:

The identification of microorganisms present in smears of aphthous ulcers and scrapings of non aphthous group were not done by culture or Polymerase Chain Reaction (PCR).

INFERENC:

Gram positive cocci and bacilli and Gram negative cocci and bacilli were present in the smears of ulcerative bed indicating presence of superimposed bacterial infection in these lesions.

Culture and Polymerase Chain Reaction (PCR) of the ulcer smear can be done to find out the microorganisms actually related to RAS.

REFERENCES