

The Impact of Sex differences and Oxidative Stress on the Severity of Recurrent Aphthous Stomatitis in Iraqi patients

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Abstract

Recurrent aphthous ulcer (RAS) is an inflammatory condition of the oral mucosa characterized by painful, well-circumscribed, single or multiple round or ovoid ulcerations. These ulcerations' exact etiologic factor(s) are not yet understood. Aim To demonstrate the effect of sex and some oxidative stress markers (GSH, MDA) with RAS. 90 participants aged (18-35) years were recruited, divided into two groups: 60 patients (35 females, 25 males) with RAS and 30 (16 females, 14 males) healthy controls. Samples were collected from specialized dental treatment centres in Ramadi and Fallujah, Iraq. The percentage of males and females was calculated by statically calculation while. The levels of glutathione (GSH) and Malondialdehyde (MDA) were measured using enzyme-linked immunosorbent assay (ELISA). Results show that RAS significantly more prevalent in females in percentage (58.30%) with (p-value=0.008) in patients than male were in percentage (41.70%) with (p-value=0.078). As well as highly significant increase in serum levels of glutathione (12.164 ± 7.295 ng/ml in RAS patients compared to (6.938 ± 3.888) ng/ml in control with (p=0.048*) and no significant difference in Malondialdehyde levels which was (37.147 ± 29.556) nmol/ml inpatients and (33.373 ± 20.531) nmol/ml in control group with (p=0.552ns). As well as, find highly effect of females compared to male in serum levels of GSH and MDA of patients which was (15.35 ± 10.323) ng/ml at (p=0.04*), (44.03 ± 31.146) nmol/ml at (p=0.03*) in female respectively. Measurements of the levels of some antioxidant such as GSH in serum suggested that these markers contributed to measure effect of oxidative stress on RAS pathogenesis and are potential markers for diagnosis and treatment. As well as, the levels of GSH and MDA highly effected by sex type.

Introduction

Recurrent Aphthous Ulceration (RAS), which is characterized by painful circular, shallow ulcers with a yellowish-grey pseudomembranous center and a well-circumscribed erythematous periphery, is one of the most common inflammatory ulcerative disorders of the oral mucosa. A burning feeling occurs 24 to 48 hours before the ulcers show themselves [1]. One RAU is classified clinically into three categories: minor, major, and herpetiform. Less than one centimeter in diameter, the tiny aphthous ulceration goes away without leaving any scars after two weeks. In contrast, the major aphthous ulceration, which is bigger (1-3 cm in diameter), takes 2-6 weeks to heal and leaves scars. Cooke's ulcers, also known as

herpetiform aphthae, are tiny, measuring 1-3 mm in diameter, and they can recur up to 100 times [2].

Anemia, bacterial and viral antigens, poor eating habits, sleep deprivation, psychological factors, genetic predisposition, trauma, nutritional deficiencies, hormone imbalance, and certain immune diseases (Behcet's disease, Crohn's disease, pharyngitis, and adenitis syndrome) have all been found to be risk factors for All of these conditions listed above are believed to have the ability to upset the equilibrium either directly or indirectly. between human antioxidant and oxidant systems, which could potentially lead to oxidative stress by combining the Reactive oxygen species (ROS) accumulate in excess, maybe making harmful interactions happen more quickly together with the harm to human cell tissue [3]. In biological systems, reactive oxygen radicals are the most prevalent type of free radical. When intracellular quantities of free radicals beyond physiological thresholds, oxidative stress takes place. This leads to lipid peroxidation, DNA and protein damage, enzyme oxidation, and the production of proinflammatory cytokines, all of which cause cell damage. Enzymatic and nonenzymatic antioxidant defense systems have been established by mammalian cells to guard against oxidative stress-induced damage [4].

Glutathione is a physiologically active tripeptide consisting of glutamate, cysteine, and glycine, glutathione is found in biological samples in both its reduced and oxidized forms, which together make up the glutathione redox pair [3]. All living things use reduced glutathione (g-L-glutamyl-L-cysteinyl-glycine, or GSH), a key intracellular nonprotein physiological antioxidant for reactive oxygen species (ROS) detoxification, because it contains the highest quantity of sulfhydryl groups [5]. It has been established that a lack of GSH may be linked to a number of human illnesses, such as diabetic, autoimmune, cardiovascular, and aging-related conditions [6]. Under oxidative conditions, two GSH molecules donate one electron each and convert into oxidized glutathione (glutathione disulfide, GSSG), which can be either reduced back to GSH by the action of glutathione reductase (GR) or excreted into the extracellular environment by an ATP-dependent transport system located in the plasma membrane to prevent the potentially toxic effects of intracellular excessive GSSG accumulation [7]. Malondialdehyde (MDA) is a stable end product of the peroxidation of membrane lipids by reactive oxygen species (ROS), and, thus, it is used as an indicator of increased lipid peroxidation. Interactions between MDA and membrane components result in disturbed structure and function of cell membranes[8]. However, factors such as dietary habits, climate, and genetic predispositions vary significantly across different populations, potentially impacting the pathophysiology of RAS. Therefore, research on RAS biomarkers in the Middle East, particularly in Iraq, is crucial due to the unique genetic and environmental factors that may influence biomarker expression in this region.

The present study aims to estimate levels GSH and MDA in the serum of RAS-affected Iraqi patients and this research is also intended to know the distribution and relation of RAS based on sex.

Materials and Methods

Study design

Case and control samples were introduced for comparison in the present study. A total of 90 participants were involved in this study and aged (18-35) years old, divided into two

groups: one consisting of 60 individuals diagnosed with RAS and the other comprising 30 healthy control subjects. This comparative design was chosen to evaluate the differences in immunological and haematological parameters between those affected by RAS and healthy controls. RAS samples were collected from specialised dental treatment centres in Ramadi and Fallujah cities. The collection of blood samples started between October 1st, 2023 – June 20th, 2024. The Research Ethics Committee of the University of Anbar/College of Medicine accepted the study protocol (Reference No: 22 on February 3rd, 2024) in a complaint with the Helsinki Declaration for Human Study.

Inclusion criteria

All participants in this study were aged between 18-35 years old. All patients had previously been diagnosed with RAS by specialised dentists. Patients had to be infected with RAS more than once a year.

Exclusion criteria

Smokers or individuals with a recent history of acute inflammation, systemic disease, Bechet's, Crohn's, celiac diseases, anaemic patients or had a vitamin deficiency, any periodontal disease, and were not pregnant.

Sample collections

Before blood samples were taken, each patient and control participant underwent a thorough interview to gather information on factors such as sex, age, number of RAS infections in the past year, weight/height, and smoking status. After this detailed assessment, informed consent was obtained from each participant prior to their inclusion in the study. Blood samples (5 mL) from each patient and control were drawn by venepuncture using a disposable syringe, which was divided into two aliquots: 1 mL was transferred into an EDTA tube for the CBC test, mixed gently for 2 minutes to prevent clotting, and then processed for the CBC analysis. The remaining 4 mL was transferred into a gel tube, allowed to stand at room temperature (25 °C) for 15 minutes to form a clot, and centrifuged at 5000 rpm for 10-15 minutes to separate the serum. The separated serum was maintained in white tubes (split into 2 aliquots) and kept at -20 °C until the time of use. The body mass index (BMI) was calculated for all participants using weight to height ratio (kg/m^2).

Laboratory tests

The serum concentrations of GSH and MDA in each patient and control were measured using enzyme-linked immunosorbent assays (ELISA). Specific kits used for these measurements included the Human glutathione (GSH) ELISA kit (Cat. No. E1462Hu) and Human malondialdehyde (MDA) ELISA kit (Cat. No. E1371Hu). All ELISA kits were procured from Sunlong Biotechnology lab company in China.

Statistical analysis

"IBM SPSS Statistics for Windows, version 22" was used to perform statistical analysis of data. Data were presented as mean $\pm$ standard deviation (SD) for all parameters. Analysis of t test was used to significantly compare means of parameters statistically. The application of Excel 2010 was used to arrange data before conducting statistical analysis.

Results The results of present study that depending on sex showed an increase in the female rate by 58.30% and 53.30% in the patient and control groups, respectively, with a highly significant difference between the two groups ($p = 0.008^{**}$), and a decrease in the male rate by 41.70% and 46.70% in the patient and control groups, respectively, with no significant difference between the groups ($p = 0.078$ ns) (Table 1 and Figure 1).

Table 1: Mean $\pm$ SD of sex in the study groups

	Male %	Female %	p- value 0.05
Patients n=60)(	25(41.70%)	35(58.30%)	0.197 ns
Control n=30)(	14(46.70%)	16(53.30%)	0.715 ns
p- value 0.05	0.078 ns	0.008 ^{**}	

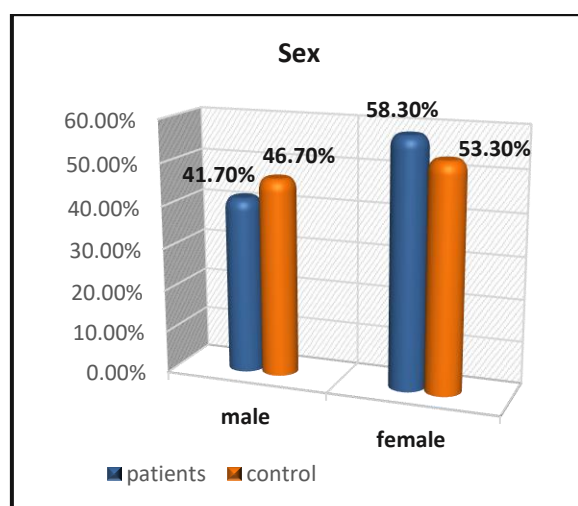
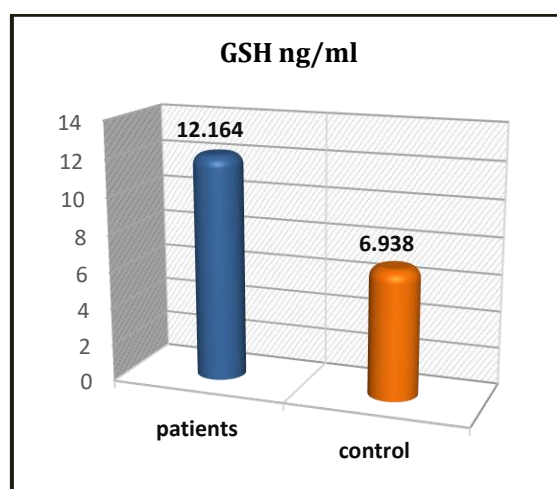
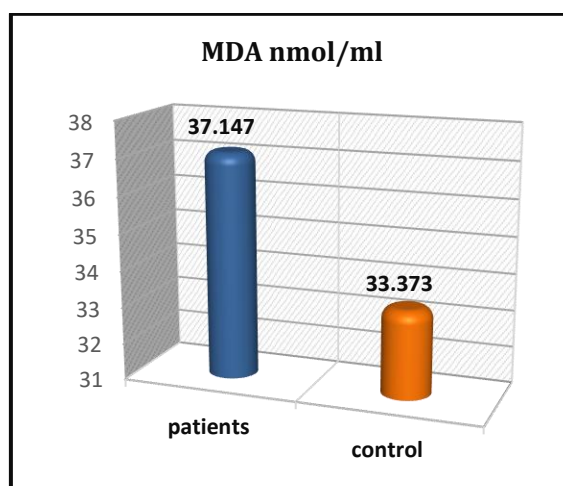


Fig 1: Mean Sex of patients with RAS patients compared with controls

Current study revealed highly significant differences in GSH and no significant differences in MDA in serum between patients with RAS and healthy controls. The mean GSH level in serum in the patient group was 12.16 ± 7.29 ng/ml in the patients group and 6.93 ± 3.88 ng/ml in the control group where there is significantly increase in $p\text{-value} = 0.048^*$ (Table 2 and Figure 2). The mean MDA level was 37.14 ± 29.55 nmol/ml in the patients group and 33.37 ± 20.53 nmol/ml in the control group where there is no significant difference between them ($p\text{-value} = 0.55\text{ns}$) (Table 2 and Figure 3).

Table 2: Mean $\pm$ SD of GSH and MDA levels in the serum of the study group

Parameters	Case	Mean $\pm$ S.D	p-value
			0.05
GSH ng/ml (mean $\pm$ S.D)	Patients n=60)(Control (n=30	12.164 $\pm$ 7.295 6.938 $\pm$ 3.888	0.048*
MDA nmol/ml (mean $\pm$ S.D)	Patients (n=60) Control (n=30)	37.147 $\pm$ 29.556 33.373 $\pm$ 20.531	

**Fig 2:** GSH levels in serum of RAS patients compared to control group**Fig 3 :** MDA levels in serum of RAS patients compared to control group

The results of effect of sex on levels of some oxidative stress parameters (GSH and MDA) find highly significant deference in levels of these parameters between male and females.

The results show highly significantly increase in GSH levels in females in mean 15.35 $\pm$ 10.323 compared to male 7.69 $\pm$ 3.442 with (p-value=0.04*) as show in table 3 and figure 4. As well as, the results show highly significantly increase in MDA levels in females in

mean 44.03 ± 31.146 compared to male 27.50 ± 19.637 with (p -value=0.03*) as show in table 3 and figure 5.

Table 3: Mean $\pm$ SD of effect of sex on GSH and MDA levels in the serum of the patients group

Parameters	GENDER (mean $\pm$ S.D)	N (Total 60)	Mean $\pm$ S.D	p- value 0.05
GSH ng/ml	Female	35	15.35 $\pm$ 10.323	0.04*
	Male	25	7.69 $\pm$ 3.442	
MDA nmol/ml	Female	35	44.03 $\pm$ 31.146	0.03*
	Male	25	27.50 $\pm$ 19.637	

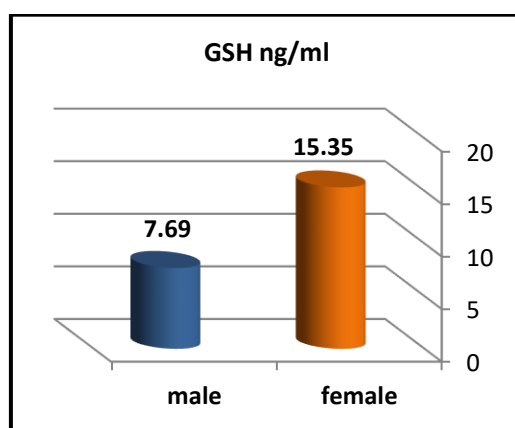


Fig 4: Effect of sex type in GSH levels in serum of RAS patients

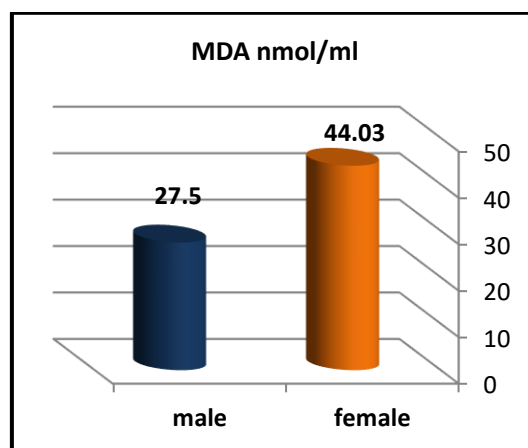


Fig 5: Effect of sex type in MDA levels in serum of RAS patients

Discussion

Our study demonstrated there were differences in RAS prevalence according to gender because the results show it was more prevalent in females compared to males in the groups of our study. Our study also demonstrated there was a significant increase in GSH levels in serum RAS-affected individuals compared with healthy individuals, with no significant differences observed in MDA levels.

Recurrent aphthous stomatitis (RAS) is the most common inflammatory ulcerative condition of oral cavity. Despite the broad research, the aetiology of RAS remains obscure. Several factors seem to have a contribution in the aetiology and pathogenesis of RAS. Some etiologic factors may have a direct or indirect effect on oxidant/antioxidant system [9]. The role of antioxidants in the maintenance of health and chemoprevention of disorders and diseases has received great attention. A possible relationship has been suggested between inflammatory processes and free radical metabolism. Immune defects can disturb the oxidant/antioxidant balance of the organism and can accelerate the formation of free radicals. The cytotoxic effects of free radicals lead to cell damage and increase oxidative stress [8].

In our study, we recorded a significant increase in RAS in females (58.30%) compared to males (41.70%). This indicates that RAS is highly significant and more common among females with regards to female predisposition to RAS. Consequently, some authors have suggested that this association is related to hormonal rates [10,11]. A minority of women with RAS have cyclical oral ulceration in conjunction with the luteal phase of the menstrual cycle [12], and a decrease in its incidence during pregnancy, thereby relating the RAS episodes to progesterone levels [13_ 15].

In current study, we recorded a highly significant increase in GSH levels in serum and a highly significant increase in serum of patients with RAS compared to control. Our findings included high levels of GSH in serum samples this support the effect of oxidative stress, our results support by previous study done by [16] which demonstrated by the increased TOS (total oxidant status) in RAS. However, this results not agree with Zhang demonstrated that the levels of GSH, determined by high-performance liquid chromatography, in patients with RAS were significantly lower than in healthy controls with increased levels of GSSG in patients of RAS [2].we suggest The high level of glutathione may be due to it being an antioxidant compound, and the higher the oxidative stress, the more it is produced in an attempt to combat these radicals, as it is considered a master antioxidant, as it directly combats free radicals and also contributes to the renewal of other types of antioxidants such as vitamin C. The size of the sample may also play a role in explaining this result, as perhaps in the case of larger samples we may notice a different result.

current study finds there is no significant difference in MDA levels between patients and control group. Results of our current study agree with results of previous study we found that the serum and salivary level of MDA (indicator of lipid peroxidation) do not have a significant difference in RAS patients compared to the control group [17]. Moreover, it is widely accepted that imbalance between free radicals and antioxidant system causes many inflammatory oral pathologies. While several previous studies find increase in levels of MDA in RAS patients compared to healthy controls such as Ziaudeen, S., & Ravindran [18] which found highly significant increase in MDA levels in patients with $p=0.05$ as well as study of [19]

also found increase of MDA levels in patients and they suggest the role of increased of free radicals cause the excessive oxidative damage and rising the MDA levels as a result. It appears that environmental and acquired circumstances, rather than genetic and hereditary variables, affect the body's level of oxidants and antioxidants, which could account for the findings.

Furthermore, the results of current study show effect of sex on levels of oxidative stress parameters. our results find highly effect of females in serum levels of GSH and MDA in RAS patients group with highly significant difference compared to male. This approved there was correlation between GSH and MDA female hormones, and this agree with previous study which find the antioxidant potential of various steroid hormones has been evaluated and it was shown that estrogens were natural antioxidants [20] . other study find observed that MDA showed positive correlation with LH and FSH while a negative correlation with estradiol and progesterone was observed [21]. These findings suggest that antioxidant genes are, almost in part, under the control of sex hormones and female hormones special , and that pathophysiology of the difference in gender disease may depend on the redox biology [22].

Conclusions

This study shows a strong correlation between RAS disease and females hormones. As well as significant alterations in GSH serum levels and RAS patients compared to control group and no alterations between RAS and serum levels of MDA. This further highlights the complex correlation between inflammation and oxidative stress. Moreover, this study enhances our understanding of the roles of female hormones and levels of (GSH,MDA) and these RAS parameters and identifies potential biomarkers that could serve as targets for future diagnostic and therapeutic strategies.

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تأثير الاختلافات الجنسية والإجهاد التأكسدي على شدة قرحة الفم القلاعية المتكررة لدى المرضى العراقيين

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البحث مستل من رسالة ماجستير الباحث الأول

الخلاصة:

القرحة القلاعية المتكررة (RAS) هي حالة التهابية تصيب الغشاء المخاطي للفم وتتميز بتقرحات مؤلمة محددة المعالم، مفردة أو متعددة، مستديرة أو بيضاوية الشكل. لم يتم فهم العوامل المسببة الدقيقة لهذه التقرحات بعد. الهدف: إظهار تأثير الجنس وبعض علامات الإجهاد التأكسدي (MDA، GSH) على RAS. تم تجنيد 90 مشاركاً تتراوح أعمارهم بين (18-35) عاماً، مقسمين إلى مجموعتين: 60 مريضاً (35 أنثى و25 ذكراً) يعانون من RAS و30 (16 أنثى و14 ذكراً) من الضوابط الأصحاء. تم جمع العينات من مراكز علاج الأسنان المتخصصة في الرمادي والفلوجة بالعراق. تم حساب النسبة المئوية للذكور والإناث بطريقة حسابية إحصائية بينما تم قياس مستويات الجلوتاثيون (GSH) والمالونديالدهيد (MDA) باستخدام اختبار المتميز المناعي المرتبط بالإنزيم (ELISA). تظهر نتائجنا أن RAS أكثر انتشاراً بشكل ملحوظ في الإناث بنسبة مئوية (58.30%) مع (القيمة الاحتمالية = 0.008) في المرضى مقارنة بالذكور بنسبة مئوية (41.70%) مع (القيمة الاحتمالية = 0.078). وكذلك زيادة كبيرة في مستويات الجلوتاثيون في مصل الدم (7.295 ± 12.164) نانوغرام / مل في مرضى RAS مقارنة بـ (3.888 ± 6.938) نانوغرام / مل في المجموعة الضابطة مع (القيمة الاحتمالية = 0.048) ولا يوجد فرق كبير في مستويات مالونديالدهيد والتي كانت (29.556 ± 37.147) نانومول / مل في المرضى الداخليين و (20.531 ± 33.373) نانومول / مل في المجموعة الضابطة مع (القيمة الاحتمالية = 0.552 ns). وكذلك، وجدنا تأثير كبير للإناث مقارنة بالذكور في مستويات MDA و GSH في مصل الدم للمرضى والتي كانت (10.323 ± 15.35) نانوغرام / مل عند (ص = 0.04)، (31.146 ± 44.03) نانومول / مل عند (ص = 0.03) في الإناث على التوالي. تشير قياسات مستويات بعض مضادات الأكسدة مثل GSH في المصل إلى أن هذه العلامات تساهم في قياس تأثير الإجهاد التأكسدي على مسببات مرض RAS وهي علامات محتملة للتشخيص والعلاج. بالإضافة إلى ذلك، فإن مستويات MDA و GSH تتأثر بشكل كبير بنوع الجنس.

معلومات البحث:

تاريخ الاستلام: 2025/01/08

تاريخ التعديل: 2025/02/10

تاريخ القبول: 2025/02/12

تاريخ النشر: 2025/09/30

الكلمات المفتاحية:

الجلوتاثيون (GSH)؛ مالونديالدهيد

(MDA)؛ الذكور والإناث؛ قرحة الفم

القلاعية المتكررة (RAS)؛ المؤشرات

الحويصلة للإجهاد التأكسدي

معلومات المؤلف

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