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Evaluation of levels of oxidants and antioxidants stress markers in saliva and serum of patients with recurrent aphthous stomatitis (RAS)

Dunia W. Sabea

Teaching staff; Faculty of Dentistry/Aliraqia University/Iraq
email: Dunia_sabea@aliraqia.edu.iq

ABSTRACT

Background:
Recurrent aphthous stomatitis (RAS) still the most collective aching oral complain with uncertain etiopathology. Saliva is the promising noninvasive diagnostic media used in a lot of biomedical research for investigation and prognosis in many oral and systemic diseases.
Aim of the study:
The present study aimed to assess the interference of most (oxidative and antioxidative) stress factors in both plasma and slaver of group with RAS and of healthy controls and compare between them inside the same patient's group.
Materials and methods:
Thirty patients with (RAS) their age was (18-55) years old were compare to other thirty (30) healthy controls matching them in age and gender were included in this study. Samples from serum and saliva were taken. Evaluation of both oxidants Malondialdehyde (MDA); and antioxidants; superoxide dismutase (SOD); catalase (CAT); total Glutathione (GSH) and Uric acid (UA) parameters was done. Statistical analysis was done for assessments' parameters.
Results:
The mean age of patients was (36.53±10.13). Oxidative stress marker was expressively greater in patients than in healthy in both serum and saliva, and in serum more than in saliva within the same RAS group. Measurements of antioxidants markers where considerably greater in serum of healthy than in the illness's group, and inside same group were more in saliva than in serum.
Conclusions:
Oxidants and antioxidants parameters play a role in RAS pathogenesis, some of them more than the others with interference not completely clear therefore more clinical studies are required.

Keywords: oxidative stress; aphthous ulcer; saliva

Correspondence:
Dunia W. Sabea
Teaching staff; Faculty of Dentistry/Aliraqia University/Iraq

*Corresponding author: Dunia W. Sabea email-address: Dunia_sabea@aliraqia.edu.iq

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is the greatest collective type of soreness illness of the oral mucosa with unclear etiology. The proven representation of RAS is described by periodic incidences of lonely or numerous aching sores devoid of relationship with general sicknesses. It is characterized by slight, rotund or ovoid sores with bounded borders, red auras and creamy or steely bases [1-3]. It's usually associated with hereditary, nutritional, infectious and psychological factors[4]. A connection among general oxidative anxiety and RAS stayed asked. Excessive generations of ROS may result in damage to most intracellular and extracellular components in a living organism, directly affect metabolism and are required for intracellular processes for proliferation, signal transduction, and apoptosis [5,6]. The usage of a board of parameters as an alternative of an only one offers extra helpful outcomes, decreases incorrect positive and incorrect negative results[7]. Slaver is a motivating substitute indicative body liquid, easy collected and possibility of repeated non-aggressive sampling[8]. The current study was designed to measure and compared the levels of MDA as oxidative stress marker and (SOD, GSH, CAT and UA) as antioxidant markers in plasma and slaver of RAS patients compared to healthy controls. Also between serum and saliva in the same RAS patients group.

Patients and methods:

In this a randomized, controlled prospective clinical study, ethical approval was obtained from the Ministry of Health for patients' examination and laboratory work and from Poisoning Consultation Centre (P.C.C.) at Specialized Surgeries Hospital for laboratory work. Thirty models with recurrent ulcers joining the Oral Diagnosis consulting room in the College of Dentistry/Aliraqia University and in the Department of Oral and Maxillofacial Surgery in Specialized Surgeries Hospital in the Medical City in Baghdad throughout the dated from JAN to AUG., 2020. Thirty healthy controls who matched them with age and gender were engaged after their informed agreement.

RAS patients were (20 females and 10 males) who had oral ulcers minimum triple eras a year for at least one-year period. All patients clinically were carefully chosen after a complete case sheet and clinical inspection of RAS ulcers diagnosed by the same dentist, all patients without new account of critical soreness and/or symptoms of systemic diseases or taking any

medications that effect on RAS. Patients group (n=30) with an age ranged from (18-55) years were participated and presented with acute, RAS lesions during the collection of serum and saliva samples. The healthy controls (n=30) stayed nominated as of the similar hospice and personal clinics, gender and age-matched patients, (20 females and 10 males) their age from (19-57) years old and didn't have any past and/or medical signs of RAS.

Saliva Samples

The subjects were asked to fast for an overnight before the morning to giving their blood and saliva samples. The study affected group were former requested to wash their oral cavities with purified water for suitable collection of saliva sample. After 5 minutes, the patients were told to be seated restfully and without oral stimulus and requested to spittle inside a disinfected elastic polyethylene pipes for 4-5 records, started to gain saliva samples to a volume of 5 ml then, were immediately placed on ice following assortment and will be separated at 3000 rpm for 10 records, these separations were stored at -80 °C till biological analyses [9,10].

Blood Samples

From each person of patients and healthy controls, venous fasting blood sample (3cc) were drawn under aseptic protocol and separated into two vacutainers, they were prepared as appropriate for different markers. Then, they were put in storage at −80°C until all testers were assessed [11].

Biochemical Assays

Assessment of (MDA)

Malondialdehyde (MDA) is a constant carbon-based complex with the insignificant formula $CH_2(CHO)_2$. A colorless liquid and is one of the finale result of the peroxidation of film fats by combative oxygen classes [12]. It is used as a mark of enlarged fatty acid peroxidation and is a pointer for oxidative stress through its reaction with Thiobarbituric acid (TBA) beneath acid state according to the (Nurten et al.,2006)[13].

Calculation of antioxidants levels:

Measurement of SOD activity:

The SOD intensity be present evaluated in models by means of the (Marklund S and Marklund G technique)[14]. Twenty parts of heparin be used to prevent coagulation of 1 ml of life blood to gain heparinized alternative. These plasma booths remained unsettled in 1.5 ml of salty solution and separated for 10 minutes at 3000 rpm. Photosensitive solidity was recorded at certain wavelength via spectrophotometer.

Measurement of Glutathione (GSH):

Is a 5, 5-Dithiobis 2-nitrobenzoic acid (DTNB) disulfide dye which is gladly react and condensed by sulfhydryl part of GSH to a deeply yellow complex. The saturation of the reactive dye is documented at 405 nm and is straightly relational to the GSH amount (Burtis & Ashwood,1999)[15].

Measurement of CAT activity:

A basic antioxidant enzyme in the tissues resistant to oxygen tension. It's a heme enzyme which is found in the peroxisome of all oxygenated cells. The CAT concentration was evaluated in models by mean implemented by Cimen MY et al.; 2003[16]. Single entity of enzyme putrefies 1 µmole of H_2O_2 in a minute at pH 7.0 at 25°C.

Measurement of Uric Acid UA (Colorimetric Kit Bio Mérieux, France)[17]

the intensity of H_2O_2 drops from 10.3 mM to 9.2 mM, the saturation lessens because of H_2O_2 withdrawal which was dignified at 240 nm via a spectrophotometer by U/mg protein. Uric acid (UA) in humanoid is the chief ending produce of purine absorption. Uric Acid is oxidized by uricase to allantoin and hydrogen peroxide, oxidized to the chromogen 4-aminoantypirine (PAP) and 3,5 dichloro-2-hydroxybezenesulfonic acid (DCHBS) forming a red-violet quinoneimine pigment as a pointer; the concentration of the recorded color (by spectrophotometry) is related towards the absorption of uric acid in the tester. This one remained deliver at a wavelength of (546 nm) UA intensities remained stated as mg/dl [18,19].

Statistical Analysis

Total statistical software analysis was done by MINTAB version 14, Pennsylvania, US. All data were written by Mean value and Standard Deviation. Use t- test for independent groups and the level of significance. t-test, S (*, *): highly significant when ($p < 0.05$; 0.01); S (*): significant when ($p < 0.05$).

RESULTS

Clinical Findings:

Thirty (30) patients complaining of (RAS) were included in this study, twenty (20) females (66.66%); and ten (10) (33.33%) were males with an age ranged from (18-55) years; the mean age for all patients was (36.53±10.13) and female/male ratio was (2:1). The same for healthy control group but age ranged from (19-57) years, with mean of all controls:(37.83±10.41) and female/male ratio was (2:1).

Clinical parameters (number of ulcers with their types and sites) that recorded on examination of thirty (30) RAS patients was shown in table 1.

Clinical parameters	No. of RAS patients 30	P -Value	Decision
No. of Ulcers	Sample	Proportion	
Single	21	0.700000	
Multiple	9	0.300000	
Total	30	1.000000	

		P= 0.001	Significant
Type of Ulcers			
Minor	**24**	**0.800000**	
Major	**6**	**0.200000**	
Total	**30**	**1.000000**	
		P= 0.000	**Significant**
Site of Ulcers			
Non-Keratinized mucosa	**22**	**0.733333**	
Keratinizes mucosa	**8**	**0.266667**	
Total	**30**	**1.000000**	
		P= 0.000	**Significant**

Table-1 Showed significance between findings of each clinical parameters. P < 0.05; 0.01

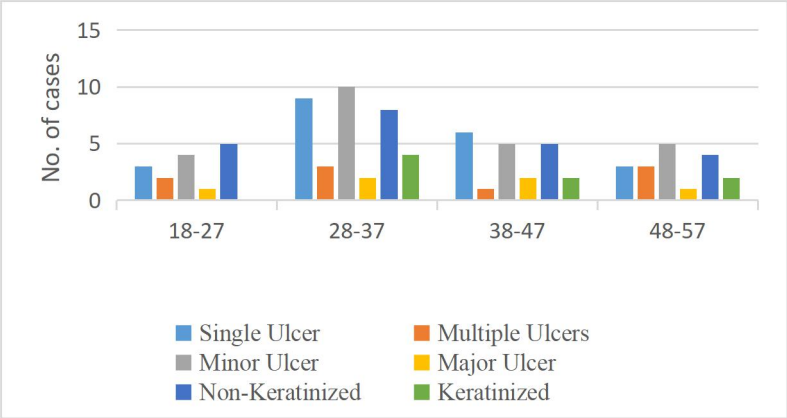

Figure-1 Showed distribution of clinical parameters of ulcers related to age.

Biochemical findings:

Assessment of Oxidative Stress Marker Malondialdehyde (MDA) level: According to (MDA) in both serum and saliva it's level was highly significant (4.79±0.71 nmol/L) and (1.16±0.22 nmol/L) correspondingly (p < 0.05;0.01) in RAS patients than that of healthy controls which were less (3.87±0.53; 0.68±0.14 nmol/L) in plasma and saliva respectively. For the patients group it's highly significant in serum when compared with the slaver of the same group, as shown in table-2.

Assessment of Antioxidants Markers (SOD; GSH; CAT and UA) levels:

SOD levels: The serum SOD level were highly significant observed (S*'*) in control group than in patients (1412.4±177.5; 1107.3±184.9) U/mg protein respectively while its level in saliva was highly significant in patients than in controls (1.64±0.19; 0.989±0.45 U/mg protein) in (p< 0.05;0.01), also a significant increase (S*'*) was recorded in SOD level in saliva of the patients group when compared to its level in the serum of the same group (1.64±0.19; 1107.3±184.9) U/mg protein in (p <0.05;0.01) as shown in table-2.

Glutathione (GSH) levels: In both serum (6.10±1.35; 3.82±1.88 µmol/L) and saliva, (2.95±0.87; 1.58±0.27 µmol/L) the (GSH) level was significantly high in control healthy persons than in the RAS patients respectively. While in the same RAS patients group its level in serum was high significant (S*'*) in (p <0.05; 0.01) than that measured in the saliva, (3.82±1.88; 2.95±0.87µmol/L) as its shown in table-2.

CAT levels: No, notable variance (p >0.05; 0.01) remained recorded in the CAT serum intensities between RAS patients and controls (187.83±10.8; 191.39±14.85 U/mg protein) respectively. While in the saliva the CAT levels was significant S (*) for (p <0.05) in RAS patients than in the controls (1.0095±0.19; 0.902±0.14 U/mg protein). When compare between CAT levels in serum and saliva (187.83±10.8; 1.0095±0.19 U/mg protein) respectively in the RAS patients group, it was highly significant (S*'*) in (p <0.05; 0.01) in serum than that measured in the saliva.

UA levels: In serum the levels of UA were (3.20±0.84; 3.72±0.74 mg/dl) in RAS patients, control group respectively with significant level in controls, when (p <0.05). However, salivary UA levels were (3.97±1.87; 2.35±1.12 mg/dl) in RAS patients, control group

respectively with high significant level in RAS patients, in (p <0.05; 0.01). When compare UA level between serum and saliva (3.20±0.84; 3.97±1.87 mg/dl) respectively for the RAS patients, UA was observed to be significantly different in saliva (p <0.05) as shown in table-2.

Plasma	MDA (nmol/L)	SOD (U/mg protein)	GSH (µmol/L)	CAT (U/mg protein)	UA(mg/dl)
RAS(n=30)	4.79±0.71	1107.3±184.9	3.82±1.88	187.83±10.8	3.20±0.84
Control (n= 30)	3.87±0.53	1412.4±177.5	6.10±1.35	191.39±14.85	3.72±0.74
p- value	0.000	0.000	0.000	0.293	0.014
Decision	S(*,*)	S(*,*)	S(*,*)	N.S	S(*)
Saliva	MDA (nmol/L)	SOD (U/mg protein)	GSH(µmol/L)	CAT (U/mg protein)	UA (mg/dl)
RAS (n = 30)	1.16±0.22	1.64±0.19	1.58±0.27	1.0095±0.19	3.97±1.87
Control (n = 30)	0.68±0.14	0.989±0.45	2.95±0.87	0.902±0.14	2.35±1.12
p-value	0.000	0.000	0.000	0.015	0.000
Decision	S(*,*)	S(*,*)	S(*,*)	S(*)	S(*,*)
Plasma & Saliva	MDA (nmol/L)	SOD (U/mg Protein)	GSH(µmol/L)	CAT (U/mg Protein)	UA (mg/dl)
Plasma RAS (n=30)	4.79±0.71	1107.3±184.9	3.82±1.88	187.83±10.8	3.20±0.84
Saliva- RAS (n=30)	1.16±0.22	1.64±0.19	1.58±0.27	1.0095±0.19	3.97±1.87
p- value	0.000	0.000	0.000	0.000	0.046
Decision	S(*,*)	S(*,*)	S(*,*)	S(*,*)	S(*)

Table-2 shows the biochemical analysis of all markers in (RAS) patients and controls. t-test, S(*, *): Highly Significant (p <0.05; 0.01); S (*): Significant (p < 0.05); Non-Significant (p > 0.05; 0.01)

Discussion

According to our knowledge this is the first study including all these different oxidants and antioxidants parameters, enzymatic and non-enzymatic stress indicators and prepared by two different hemolytic and no hemolytic preparations, also we did comparison and try to create link between serum and saliva in the same RAS patients group to evaluate concentration of the different indicators in two different fluid media within the same patient.

Clinical Findings Analysis

The RAS patients mean age in this study was (36.53±10.13) years, this was earnings that RAS remained initiate about the 4th. span. This will agree with (Ship, 1996 and Oh et.al. 2006) [20,21] whom the most affected age in their studies was the 4th. decade but disagree with (Safadi, 2009; Akoglu et.al. 2013; Essa and Zaidan, 2013). [22-24] who found age was around 3rd. decade this may be due to psychological and mentality of these age groups (3rd, 4th) towards life with their effect on their immune system, lead to RAS ulcers more than other age group people. According to number of appearing ulcers this study showed (70%) of patients with single ulcer whereas the remaining were multiple, and this in agreement with (Cu et. al., 2016) [25]. Also (80%) were minor ulcers and (20%) were major, this will agree with (Neville et al., 2008 and Greenberg al., 2008) [26,27], but disagree with (Mohammad, 2012)[28]. In this study no patient was with herpetiform type ulcers. Most of these ulcers about (73.33%) appeared on the non-keratinized oral mucosa and the remaining on keratinized mucosa this is due to fragile, soft areas and movable structure so, less tough with commonly exaggerated via distress which was the more triggering cause in evolving RAS. Our results come to an agreement with (Scully et. Al., 2003; Cawson& Odell, 2008 and Tappuni et.al. 2013). [29-31]

Biochemical Findings Analysis

Reactive oxygen radicals may lead to destroy normal cell structures and functions. Many studies discuss imbalance between oxidative stress and antioxidative defense system in oral mucosa.

Oxidative stress marker MDA which is single indicator mostly used for fatty acids peroxidation, it's harmful consequence results to alterations of, adjustments of amino-acid sequence, and fat building. Impaired antioxidants defense mechanism has led to increase activation of peroxidation reactions which cause cell membrane damage. That's explain the increase in MDA intensities in both serum and salivate of RAS group significantly more than healthy control persons. This is agreeing with (Saral et.al. 2005; Altinyazar et.al. 2006; Gurel et.al. 2007) [32-35]. Serum (SOD) level was highly significant in controls than in RAS patients even when compare its level in serum and saliva of the same RAS patients, there was significant increase in SOD salivary level, this can be logically due to that when RAS ulcers starting to appear in the oral cavity, this may be recruited all antioxidants molecules to concentrate

more and more in the saliva where the ulcers present as an indication of the local reaction of the body against increased oxidative stress, this was agreeing with (Momen et al. 2010; Saxena, 2011; Valko et.al. 2007; Maciejczyk 2019). [36-39] While other recorded the opposite [40-44].

Glutathione (GSH) is a main element in body defense structures, plentiful non-protein-conjugated thiol-enclosing complex establish in chambers, by intracellular concentration acting as a detoxifying agent. This study showed significant high levels of GSH in serum and saliva of the healthy controls more than RAS patients, this was disagreeing with many previous studies which found GSH either increase in serum and decrease in saliva or contradictory results. [16,39] This increase of an important antioxidant molecule in plasma and saliva of healthy persons and its reduction in RAS patients confirmed the important role of GSH non-enzymatic protection against radical species. Genomic and organic proof has verified that glutathione and glutathione-reliant on enzymes show a crucial character in the cellular guard compared to lethal ecological means (Smith et al.,1996 and Gavriliuc et al., 2007) [45,46]. GSH significantly increasing level in the serum of RAS patient more than in his saliva, may be explained by that when free radicals increase, antioxidants are consumed for the defense that detoxifies the harmful particles leading to reductions of beneficial status. GSH is cataloged at several levels and this section leading to less concentration level in saliva of the patient (Young and Woodside, 2001; Turell et al., 2013; Arikan et al., 2009 and Sardesai, 1995) [47-50].

The levels of CAT in this study were non-significant in the plasma of the RAS group and controls, but, obviously found in their saliva. Catalase has been predictable as the minor defending enzyme it hydrolyses H_2O_2 into H_2O and O_2 and not acts unless reaching an ideal absorption of hydrogen peroxide this in explain why it's level was not significant in the serum of the patients, and significant in their saliva which is the local media of the RAS ulcers and there was higher level of hydrogen peroxide formation. This was agreeing with (Momen et al., 2010; Saxena 2011, and Jesija et al.,2017) [36,37,51]. Level of CAT in the serum was highly significant than saliva, this is due to persistent acute effect of RAS ulcers, lead to increased antioxidant status, but long term effects can reduce it and became less in the saliva within time and their level was increase in serum. Results of UA in this study showed significant level in the serum of controls, while in saliva, it was highly significant in RAS patients. This can be easily clarified through previous studies reports which established that UA stands the greatest chief non enzymatic antioxidant also intended for around 70% of the entire resistant oxidant bulk in salivate. So, UA level is less in serum of RAS patients because it was directed towards saliva where oxidative stress increased locally and RAS ulcers appeared and there is a need for antioxidants molecules activity. When we compared UA level in the patients' group, it was significant high in saliva more than in the serum because UA is the strongest particle against free radicals and is a hunter of solo oxygen and free particles in addition to its action to prevent the fatty acid deprivation from peroxidation so, its level in saliva was more than in serum, these results are similar to others (Ames et al.,1981; Cimen et al., 2003). [52,16] although, there is another theory about a dual role that UA plays if is it the oxidant radical? or is it active part in antioxidant system? (Duk-Hee Kang and Sung-Kyu Ha, 2014). [53]

CONCLUSION

Recording levels of both oxidative and antioxidative stress by measuring their indicators in important fluid media (saliva and serum) in diseased and healthy persons is important not just to understand the etiopathology and the mechanism but also to found the suitable treatment. If the reduction in antioxidant molecules lead to RAS ulcers this reduction surely not cause only RAS but many other diseases which the accused individual complain from. So, when there is an episode of RAS for a reason or another there should be an imbalance between total (oxidant and antioxidant) stress. As first step to solve this puzzle we need to understand the molecular changes occur and lead to this popular oral condition.

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