OXIDATIVE STRESS IN PREGNANT WOMEN WITH GINGIVITIS AND DENTAL CAVITIES

CAMELIA DALAI[1], C. DALAI[2], IOANA IGNAT ROMANUL[3], OTILIA MICLE[4], MARIANA MUREȘAN[5], LIANA ANTAL[6], F. BODOG[7]

[1,2,3,4,5,6,7]*University of Oradea*

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Abstract: *Introduction: ROS production is inevitable in all aerobic organisms including in the human body which posses a complex antioxidant defence system. There is a balance between ROS and antioxidants. If this balance is disrupted in favour of ROS, an oxidative stress situation is created.(20) Purpose: The aim of this study was to evaluate the oxidative stress markers in serum and saliva of pregnant women with gingivitis and dental caries and to investigate the use of simple methods such as the determination of some antioxidants such as ceruloplasmin and uric acid for the clinical monitoring of the dental diseases and treatment. Materials and methods: Our research was carried out on 27 normal pregnant women aged between 18 and 39 years-old, in the fifth to ninth month of pregnancy, selected among the patients attending the Obstetrics and Gynaecology Clinics of Oradea. The results were compared with a control group matched with the study groups in terms of age, BMI, blood pressure. For proving the oxidative stress, we established the level of malondialdehyde using a method with thiobarbituric acid (TBA) and the level of ceruloplasmin with the Ravin method. The supplementary measurements such as uric acid, iron and CRP were done on HITACHI 912 Roche Diagnostics and using reagent Greiner Diagnostic, Germany. Results and discussions: The salivary MDA was higher in both studied groups compared to the reference one. We found the ceruloplasmin concentration in saliva lower in pregnant women with dental caries or with gingivitis in comparison to the control group. No significant differences were registered between the control group and pregnant women in salivary and serum uric acid concentration. CRP was immeasurable in all saliva samples, the values obtained were below the analytical range (<0.8mg/l). Conclusions: The indicators of oxidative aggression may provide a tool for monitoring the oral health of the pregnant women.*

Cuvinte cheie: *gingivită, carii dentare, gravidă*

Rezumat: *Introducere: Producerea de oxidanți este inevitabilă în organismele aerobe inclusiv în corpul uman care deține un sistem antioxidant complex de apărare. Există un echilibru între oxidanți și antioxidanți. Dacă acest echilibru este modificat în favoarea oxidanților atunci apare stresul oxidativ.(20) Scopul studiului: Scopul acestui studiu a fost de a evalua markerii stresului oxidativ în serul și în saliva femeilor gravide cu carii dentare și gingivită și de a investiga utilizarea de metode simple de laborator de determinare a unor antioxidanți precum ceruloplasmina, acidul uric în evaluarea clinică și tratamentul afecțiunilor dentare. Material și metodă: Cercetările noastre au fost efectuate pe 27 de femei gravide sănătoase cu vârsta cuprinsă între 18 și 39 ani, în a cincea și a noua lună de sarcină, selectate dintre pacientele Spitalului de Obstetrică-Ginecologie, Oradea. Rezultatele au fost comparate cu un grup de control asemănător cu grupul de studiu privind vârsta, IMC (index de masă corporal), tensiunea arterială. Pentru demonstrarea stresului oxidativ am dozat concentrația malondialdehidei utilizând metoda cu acid tiobarbituric (TBA) și a ceruloplasminei cu metoda Ravin. Analize suplimentare precum acidul uric, fierul și proteina C reactivă s-au făcut pe analizorul HITACHI 912 Roche Diagnostics și folosind reactiv Greiner Diagnostic, Germania. Rezultate și discuții: MDA salivară a fost mai mare în ambele grupuri studiate, comparativ cu grupul martor. Concentrația salivară a ceruloplasminei este mai mică la gravidele cu carii dentare sau cu gingivită, în comparație cu grupul de control. Nu am obținut diferențe semnificative ale concentrației acidului uric atât în ser cât și în salivă la gravide comparativ cu grupul de control. Valorile proteinei C reactive în salivă s-au aflat sub limita de detecție a metodei (<0.8mg/dl). Concluzii: Indicatorii agresiunii oxidative dozați pot oferi un instrument de monitorizare a stării de sănătate orală a gravidelor.*

INTRODUCTION

Inflammatory periodontal diseases affect 10% to 15% of the world's population and are a major cause of tooth loss in adults.(1,2) In the pregnant women, the frequency of gingivitis ranges from 35% to 100% and grows during the second trimester.(3) Researches also showed that resolution of gingival inflammation occurred when pregnant women were taught correct oral hygiene procedures.(4) The accumulation of active progesterone and estrogen in gingival tissues may determine a higher vascular permeability and enhance bacterial growth, providing essential bacterial growth factors (vitamin K).(5 6,7,8)

[1]Corresponding author: Dalai Camelia, Str. Privighetorii, Nr. 18, Oradea, România, E-mail: cameliadalai@gmail.com, Tel: +40740 236973
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Some research suggested that gingivitis and periodontitis seem to be independent risk factors for preterm birth/low birth weight.(9,10,11,12,13,14) Periodontal infections may cause systemic host responses with an up regulation of serum cytokines, a possible mechanism of preterm birth.(15,16)

There are many studies which demonstrated that these pregnant women's diseases affect a predisposed group of the population that presents an exacerbated inflammatory/immune response to the pathogenic bacteria accumulated on the teeth and around the gingival tissue, which in turn lead to tissue damage.(17,18) The exacerbated response characterized by hyper inflammation fails to remove the causative pathogens and generates prolonged release of neutrophil proteolytic enzymes, proinflammatory mediators and reactive oxygen species (ROS). All of these factors cause the destruction of the periodontal attachment to root surfaces and adjacent alveolar bone.(19)

ROS production is inevitable in all aerobic organisms including in the human body which posses a complex antioxidant defence system. There is a balance between ROS and antioxidants. If this balance is disrupted in favour of ROS, an oxidative stress situation is created.(20)

PURPOSE

The aim of this study was to evaluate the oxidative stress markers in serum and saliva of pregnant women with gingivitis and dental caries. The second objective was to investigate the use of serum antioxidants such as ceruloplasmin and uric acid for the clinical monitoring of the dental disease and for the efficiency of the treatment.

METHODS

The study was carried out on 27 normal pregnant women between 18 and 39 years old, in the period of the fifth to ninth month of pregnancy, selected among the patients attending the Obstetrics and Gynaecology Clinics of Oradea. The chosen group was divided in the following groups:

(1) pregnant women with dental caries n=12

(2) pregnant women with gingivitis n=15

The results were compared to the control group:

(3) non-pregnant healthy women n =15, matched with the studied persons in terms of age, BMI, blood pressure.

Clinically, the pregnant women with gingivitis were characterised by gingival oedema, a reddish violet colour of the gingival margin, gum bleeding, spontaneously or induced by teeth brushing or mastication. At local examination, the pregnant women with dental caries were characterised by little destructions of the hard tooth tissue. Written informed consent was obtained from all participants, prior to enrolment and the study was approved by the institutional ethical committee. Maternal venous samples were collected after overnight fasting. The blood was collected in vacuum tubes. Specimens were transported to the laboratory immediately after collection, centrifuged at 1500g for 10 min to separate serum.

Unstimulated whole saliva samples were collected under resting conditions between 7.30 and 10.30 a.m. The patients sat immobile and spit into sterile plastic containers for 5 min until the saliva was accumulated in the floor of the mouth. Subjects were asked not to swallow any saliva during the collection. Saliva samples were centrifuged (centrifugal force: 1,000g) for 10 min. to remove bacteria and other external materials. The resulting clarified fluid was used for the biochemical assays. We proved the presence of the oxidative stress in terms of the level of malondialdehyde tested with thiobarbituric acid (TBA) (21) and the concentration of ceruloplasmin with the Ravin method. All biochemical parameters were measured on Hitachi 912 instrument, Roche Diagnostics.

Uric acid was measured with an enzymatic photometric test using TBHBA (2, 4, 6-tribromo-3-hydroxybenzoic acid) (Cat. No. 1 3021 99 10 704), Diasys, Germany.(9,10) We also estimated the level of serum iron with an important role in the ROS formation. The dosage of the iron was performed with a photometric test using Ferene (Cat. No. 1 1911 99 10 704), Diasys, Germany.(11) The C Reactive Protein (CRP) was assessed with an immunoturbidimetric method (Cat. No. CPTXLH00), Diagam, Belgium.(12,13)

Data are expressed as mean ± SD (standard deviation). For establishing the significance of differences, we applied the Student's test. The P values <0.05 were considered statistically significant. Pearson's correlation coefficient (r) revealed the connections between the different studied parameters (http://www.physics.csbsju.edu/stats/t-test.html).

RESULTS

The results of our investigations are presented in table no. 1. There were no significant differences in age, body weight, body height, blood pressure between the pregnant women and the non-pregnant controls at the time of recruitment (table no. 2).

Table no. 1. The values of the MDA, ceruloplasmin, uric acid, iron, CRP in pregnant women with dental caries and gingivitis

	MDA nmoli/ml	Ceruloplasmin mg/dl	Uric acid g/l	Fe µg/dl	CRP
Group 1 Serum	2.84 ± 0.84	43.75 ± 3.54	3.13 ± 0.50	102.14 ± 44.76	2.30 ± 2.44
Group 1 Saliva	1.01± 0.49	1.14± 0.51	2.81 ± 3.33	1.44± 1.35	< 0.8
P of group 1 serum compared to group1-saliva	0.001	0.0001	0.78	0.0001	
Group 2 Serum	3.07 ± 1.11	46.48 ± 6.36	3.12 ± 0.96	98.75 ± 24.65	4.21 ± 3.47
Group 2 Saliva	1.26 ± 0.6	1.92 ± 1.02	3.14 ± 1.62	2.48 ± 4.11	<0.8
P of group 2 serum compared-saliva	0.005	0.0001	0.98	0.0001	
P of group 1-group 2 serum	0.63	0.29	0.99	0.83	
P of group 1-group 2 saliva	0.48	0.32	0.75	0.16	
Control Group Saliva	0.64 ± 0.23	2.99 ± 1.70	2.63 ± 1.44		<0.8
P group 1 - control saliva	0.02	0.027	0.88		
P group 2 - control saliva	0.01	0.03	0.46		
Control Group Serum	1.91 ± 0.33	32.11 ± 1.62			
P of group 1-control Serum	0.005	0.0001			
P group 2-control Serum	0.006	0.0001			

Groups: (1) pregnant women with dental caries n=12

(2) pregnant women with gingivitis n=15
(3) non-pregnant women n =15

Table no. 2. Clinical parameters of the studied groups

	Medium	Age (years)	Body weight (kg)	Body height (m)	SBP (mmHg)	DBP (mmHg)	Age of pregnancy (weeks)
Group 1 n=12	41.66 % U 58.33 % R	30.1 ± 4.95	69.5 ± 14.69	1.65 ± 0.06	115.6 ± 13.25	70.8 ± 9.70	33.85 ± 7.60
Group 2 n=15	40% U 60% R	27.0 8 ± 4.67	74.08 ± 12.67	1,67 ± 0.03	112.08 ± 11.12	71.91 ± 10.09	25.95 ± 8.81
Control Group (n= 15)	70% U 30% R	26.0 9 ± 3.74	67.02 ± 9.80	1.65 ± 0.05	116.90 ± 9. 53	70.55 ± 8.31	-
Group 1 n=12	41.66 % U 58.33 % R	30.1 ± 4.95	69.5 ± 14.69	1.65 ± 0.06	115.6 ± 13.25	70.8 ± 9.70	33.85 ± 7.60

CRP was immeasurable in all saliva samples, the values obtained were below the analytical range (< 0.8mg/l). The salivary MDA, as a specific marker of oxidative stress was higher in both studied groups compared with the reference group **(**table no. 1). We found a low salivary ceruloplasmin concentration in pregnant women with dental caries or with gingivitis in comparison to the control group (table no. 1). No important variation of salivary uric acid concentration between the control group and both experimental groups were noticed (group 1 p=0.88, group 2 p=0.46). A very significant difference appeared between salivary and serum MDA, ceruloplasmin and iron in the studied groups (p=0.0001 table no. 1). The salivary and serum uric acid concentration in the experimental groups were alike (table no. 1).

The analysis of MDA, ceruloplasmin, iron and uric acid in serum revealed no significant differences between the pregnant women with dental caries or gingivitis (table no. 1). The same parameters in saliva of the studied groups had no remarkable variations. In comparison with the serum of the control group the level of MDA and ceruloplasmin were considerably high in the pregnant women (table no. 1).

DISCUSSIONS

The molecular oxygen inspired can be used by the cells only after its activation. The activation consists of a reduction process, which means that with each metabolic step the molecules gain one electron. So, anionic oxygen named superoxide, peroxide, and hydroxyl etc. is formed and finally, these changes lead to the formation of water.(22)

The activated oxygen inside the cell exceeds the necessities. Here, it is balanced by the intervention of antioxidant enzymes like superoxide dismutase, catalase, gluthation peroxidase. Despite this, about 2% of ROS escape from the cells and pass in to the blood. Blood possesses other antioxidants factors like vitamin E, C, ceruloplasmin, bilirubin, uric acid. The oxidative stress represents an imbalance between the ROS production and the level of the antioxidant factors. Brock et al have considered the effect of the imbalance between oxidants and antioxidants, having a damaging effects on the periodontium in the patients with periodontitis.(23) The main source of superoxide anion ($O2^-$) and other ROS responsible for the initiation of the pathological reactions is the respiratory chain. However, its presence in the periodontal tissue results from the activation of phagocytes (neutrophils and macrophages), the main cellular antibacterial agents.(24,25)

Hydroxyl radical (OH) is one of the most harmful ROS capable of damaging molecules such as DNA proteins and lipids. Hydrogen peroxide (H_2O_2), in spite of being considered a less potent ROS, is capable of crossing the nuclear membrane and also injuring the DNA.(26) The hydroxyl radical initiates a classical chain reaction known as lipid peroxidation leading to vasodilatation and bone reabsorption.(27) Wang et al suggested that the superoxide anion is involved in bone reabsorption. Studies have demonstrated the presence of this anion in the reabsorption zones adjacent to the osteoclasts.(28)

MDA is a product of breaking long carbon strings of fatty acids and it is considered as a specific marker of oxidative stress. Exaggerated postprandial surges in glucose and triglycerides result from high calorie diets and are absorbed into the bloodstream rapidly. Elevated glucose and lipid levels generated ROS at a rate that exceeds the capacity of the Krebs cycle and electron transporters of the inner mitochondrial membranes to generate adenosine triphosphate. Electron leakage leads to the single electron reduction of molecular oxygen, thus forming superoxide (O^{2-}) and, further downstream, ROS.(29) Pregnancy is a physiological state which is characterized by a high level of glucose and triglyceride in serum.

In gingivitis of the pregnant women we found a higher level of salivary iron than in pregnant women with caries but without statistical significance. Cells destruction is associated with delivery of cytocromes enzyme. The iron in the organism is present in each cell like cytocromic enzymes. The amount of cellular iron reaches about 1% of the total iron in the organism. The intervention of ferrous iron in the Fenton and Haber-Weiss reactions is a source of hydroxyl formation which stresses the oxidative aggression. There are some bacteria which need the presence of iron. These are the noxious effects of iron in saliva. The high level of salivary iron in gingivitis of pregnant women is a factor which takes part on the formation of the dangerous type of ROS (hydroxyl) and this way, it increases the oxidative stress.

The serum concentration of MDA in pregnant women is highly compared to the control group of non pregnant women. These results show that systemic oxidative stress of pregnant women is not only of salivary origin. In our study we found a high concentration of salivary MDA in both experimental groups. It is the sign of a local intraoral production probably due to the inflammation of the soft tissue.

Oxidative stress is increased in pregnant women with dental disorders, particularly due to the locally reduced concentration of ceruloplasmin. The low concentration of ceruloplasmin in saliva may contribute to enhance the damage of the gums and teeth more than the changes of it in the serum. The role of salivary ceruloplasmin consists of the reduction of the inflammatory processes initiated by the local bacterial aggressions. Systemic and local gingival fluid antioxidants level decrease in pregnancy and periodontitis. The antioxidants defence system is debilitated in the last phase of pregnancy which deteriorates the periodontal status.(30) Wei D et al in 2010 found that lipid peroxidation was higher in the periodontal region with an increased concentration of superoxide dismutase. Also, they underlined that non-surgical therapy can restore the subject antioxidant capacity modifying locally and systemically the level of MDA of the superoxide dismutase and the total oxidative status.(31) Naofumi Tamaki et al. demonstrated that a systemic increase in the oxidative stress may influence the rate of progression of periodontal disease.(32) Moore et al. suggested that urate contributes in excess to 70% of salivary total antioxidant activity.(33)

In the present study, urate was the antioxidant component in saliva almost near the control concentrations. It is not clear whether the urate content of whole saliva directly reflects plasma concentrations or only the salivary production. However, interactions between scavenging antioxidants may

occur.(34) Further studies on this problem must be conducted. Some research have proved an antioxidant depletion in periodontitis, locally, in the periodontium and also in the plasma.(35) They also found an inverse relationship between concentrations of plasma total antioxidants and vitamin C with an increased prevalence in periodontitis.(36) If one of the salivary antioxidants concentration is decreased, the another salivary antioxidants component can compensate its function.

CONCLUSIONS

1. The pregnant women with gingivitis and dental caries have a salivary oxidative stress indicated by high concentration of MDA and a low level of ceruloplasmin in saliva.
2. The concentration of salivary and serum uric acid in the experimental groups were alike.
3. In gingivitis and dental caries of pregnant women the levels of serum MDA, ceruloplasmin and iron had no significant variations.
4. The indicators of oxidative aggression may provide a tool monitoring the oral health of pregnant women.

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