Journal of Medicinal and Chemical Sciences 5 (2022) 1253-1264

Journal of Medicinal and Chemical Sciences

Journal homepage: http://www.jmchemsci.com/

Original Article

Study the Effect of Pregnancy on Oxidative Stress Status in Pregnant Women with Gingivitis

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ARTICLE INFO

Article history

Receive: 2022-05-13
Received in revised: 2022-06-04
Accepted: 2022-07-05
Manuscript ID: JMCS-2206-1535
Checked for Plagiarism: **Yes**
Language Editor:
Dr. Fatimah Ramezani
Editor who approved publication:
Dr. Azahar Ali

DOI:10.26655/JMCHEMSCI.2022.7.15

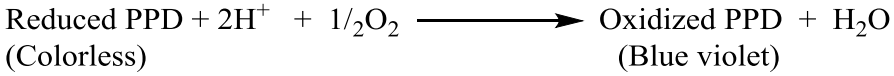
KEYWORDS

Gingivitis
Pregnancy
Oxidative stress
Total antioxidant capacity

ABSTRACT

Background: Gingivitis is an inflammation limited to marginal gingival tissues, which can be referred to as the mildest form of periodontal diseases, initial signs of inflammation which include: redness, bleeding, swelling, exudation, and to a lesser extent pain, which indicate the presence of gingivitis. Current research was proposed to detect the correlation between pregnancy status and oxidative stress (OS) levels among gingivitis pregnant women and to elucidate the role of pregnancy status in the pathogenicity of gingivitis.
Methods: The current study was conducted in the Dental Center at Thi-Qar province (Iraq), and it included three study groups: 35 pregnant women with gingivitis, 35 non-pregnant women with gingivitis, and 30 non-pregnant women without gingivitis (the healthy control), with an age balance between the three study groups. Serological and salivary levels of malondialdehyde (MDA), total anti-oxidant capacity (TAC), protein carbonyl (Pc), protein, nitric oxide (NO), and ceruloplasmin (Crp) were biochemically determined.
Results: The results of the tests showed that the concentrations of MDA, Pc, the total proteins (TP), NO, and Crp were significantly higher among pregnant and non-pregnant women with gingivitis compared with healthy controls in both serum and saliva, whereas the inverse findings were reported for TCA biomarker. The same results profiles were reported in comparison between pregnant women with gingivitis and non-pregnant women with gingivitis for serum biomarkers. For saliva biomarkers, the results revealed significant elevated levels of MDA, Pc, and NO among pregnant gingivitis women in comparison with non-pregnant gingivitis, whereas the other biomarkers revealed non-significant differences.
Conclusions: The pregnancy status had an effect on the OS level which lead to a higher level of OS in both serum and saliva and the correlation between the pregnancy status and serum and/or saliva OS levels was positive which indicated the possible utility of its present lead to gingivitis development.

GRAPHICAL ABSTRACT



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Introduction

Gingivitis is an inflammation limited to marginal gingival tissue, which can be referred to as the mildest form of periodontal disease, initial signs of inflammation which include: redness, bleeding, swelling, exudation, and to a lesser extent pain, which indicate the presence of gingivitis [1]. Periodontitis results from the extension of the inflammatory process that began in the gums to the supporting periodontal tissues. This is the result of overgrowth and differentiation of plaque bacteria [2].

Gingivitis can be treated by maintaining good oral hygiene and without the use of medicated medications, gingivitis can progress to periodontitis, where periodontitis destroys tissue and erodes the bone around the teeth. Ultimately, gingivitis can lead to tooth loss [3], while environmental factors, smoking, poor oral hygiene, poor diet, and genetic influences (family history of gum infections) affect the severity of the disease [2].

Saliva is a prominent oral watery fluid that is important for maintaining, protecting, and curing oral tissues, as well as many other roles, like aiding speech, digestion, lubrication, and the perception of taste. A normal saliva flow rate is essential for maintaining oral health and gingivae health in particular [4]. Saliva is considered as "reflecting mirror of the body", and in the latest periods, it has been wonderfully referred as a sample for screening and diagnosing disorders, monitoring conditions advancement, measuring medications magnitude, etc. because of the abundance of existing biomarkers and ease of collection. In addition, saliva has a vital role in maintaining the immune functions and as an antioxidant to defend against inevitable microorganisms and the body's general OS [5]. In fact, saliva is the primary body's line of defenses against reactive oxygen species (ROS), because certain proteins and/or lipid antioxidants are produced in the salivary glands and transferred to the lingual cavity through the blood [6].

A significant positive correlation has been observed between hormones disturbance and gingivitis condition that occur with pregnancy, as these hormonal changes increase tooth sensitivity, collagen synthesis rate, and connective tissue changes and gingiva can develop into the periodontal disease during this period [7]. Nevertheless, it can be stated that current observational researches generally verify a vigorous link between oral health problems and poor pregnancy consequences, whereas there is still some controversy [5].

All inflammatory diseases cause to increase OS, which can lead to further tissue damage, and gum tissues were not excluded, exacerbating periodontitis [8]. It is hypothesized that gingivitis and periodontal diseases may systemically burden pregnant women due to endotoxins, inflammatory cytokines, and OS of the mother and fetus. Thus, this may have a role in the pathophysiology of preeclampsia in pregnant women [9]. In the human body, a delicate balance occurs between the ROS-production and ROS-elimination. Oxidative stress represents an imbalance between oxidants and antioxidant systems, causing confirmed damage to cell physiology [10], a pathological mechanism that has contributed to many inflammatory diseases which lead to proteins, lipids, and nucleic acids destruction. Oxidative stress is known to have a huge involvement in many oral pathological conditions, like impaired salivary glands, dry mouth, gingivitis, periodontitis, precancer lesions, and oral cancer [11].

Malondialdehyde is an important biomarker for OS because it is one of the lipid peroxidation end-products that caused by an increase in levels of ROS. High levels of MDA and decreased activity of salivary antioxidants were previously reported in patients with gingivitis and periodontitis [5]. Almost all amino acids are oxidized by ROS, and thus generating hydroxyl and carbonyl groups, which change the structure and lose the functional role of the proteins. Detection of protein-bound carbonyls is a common method for the general assessment of protein oxidation, and as an indicator of oxidative damage, it has been shown to accumulate during aging and many human diseases [12]. Actually, Pc assays have become widely used and many laboratories have developed individual protocols for them [13].

Antioxidants act as a free radical cleaning to reduce OS in the body and are believed to have a role in overall safety [11]. Antioxidants act collectively, and TAC may be the most relevant biomarker for assessing the defense capabilities [14]. It can prevent or slow down the harmful effect of free radicals and promote oral and general health [1]. There are few studies on the level of antioxidants in pregnant women with gum disease [9].

Nitric oxide is one of the important mediators regulating endothelial vasodilatation and function, as it monitors the level of vasculitis, vascular contraction, and cell reproduction, and it regulates the release of various growth factors [15]. Significant protective effects occur at picomolar to nanomolar concentration levels of NO, in the case of high levels of NO and its by-products, cytotoxicity occur. The production of NO in gingival tissues is a part of the non-specific anti-bacterial protection against periodontal pathogens. Some reports have shown that a high amount of NO activity within inflamed gingival tissues [15]. Furthermore, it can also be mentioned that the differences in the synthesis of NO at the level of the gums is likely differ from its levels within the blood stream, it is antibacterial in the mouth, while more broadly affecting the function of the endothelium [15].

Ceruloplasmin is defined as a glycoprotein that contains copper and has a molecular weight of 132 Kilo Dalton. It is principally produced by the liver's cells and had multiple roles; like an antioxidant, copper transporter, and anti-inflammatories, it represents one of the acute phase proteins and pro-inflammatory activities [16]. Since Crp is an acute phase reactive, its level in the serum will increase during infection, inflammatory, and trauma due to increased gene transcription in liver cells mediated by autocytokines. In addition, it plays an essential role to decrease the levels of OS through its ability to scavenge for the free radicals (superoxide anion). According to several previous studies, Crp is an effective biomarker for several immunological conditions along with chronic gingivitis and has shown a strong association with periodontal disease activity [17, 18]. The current study was proposed to detect the relationship between pregnancy status and the OS levels among gingivitis pregnant women and to elucidate the role of pregnancy status in the pathogenicity of gingivitis.

Materials and Methods

Subjects and study design

A case control study was done which included three study groups. The first group included 35 pregnant women with gingivitis, and the second group included 35 non-pregnant women with gingivitis, whom attended the Dental Center in Thi-Qar province (Iraq), while the third control group consisted of 30 non-pregnant women and without gingivitis with the age balance between the three study groups. Gingivitis was diagnosed by physician at the center that mentioned above. A written approval was reported from each person enrolled in the current study to meet the international ethical standards for research, and the proposed research was approved by ethical consideration committee at Southern Technical University, Al-Nasiriyah Technical Institute. The current research was conducted from January to August 2021. All serological and/or salivary biomarkers tests were performed at Community Health Department/Al-Nasiriyah Technical Institute.

Subjects eligibility criteria

For the first group, women who had any of the following criteria were excluded from the current study: women with infectious diseases, chronic or autoimmune diseases, underwent corticosteroid therapy, taking any biological agent or supplement vitamins, recent surgery, and blood transfusions, whereas the women with following criteria were included: pregnant women with gingivitis, non-smokers and had none of the exclusion criteria mentioned above.

For the second group, the women with following criteria were included in this group: non-pregnant women with gingivitis and recently not taking corticosteroid therapy or vitamins supplementation, the absence of any infectious, autoimmune or chronic diseases, absence recent surgery and blood transfusions, not taking any

biological agents, had matched age with the first group, and non-smoker.

The third study group inclusion criteria were the same inclusion criteria of the second group, but the women were without gingivitis. In addition, women with even a simple infection were excluded.

Samples collection

Samples (blood and saliva) were collected simultaneously, always in the morning after 9-10 hours (hrs) of fasting (at least 6 hrs). Five milliliter (ml) of blood were collected (from each subjects) by vein puncture, and then more than 5 ml of saliva were collected (from each subjects) in a period of no more than 10 minutes. For saliva samples collection, the spit method was used in a seated position. The subject was relaxed and resting for 5 minutes before sample collection, tilting the head slightly down, and reduces the movements of the face and lips; rinse the mouth three times with distilled water. This was followed by spitting out the saliva into a clean tube. Finally, 0.5 millimolar (mM) of ethylenediaminetetraacetic acid was added for each saliva sample.

After blood samples clotting, at once by using centrifuge (Hettich, Germany), specimens were separated (saliva: 10,000 g, 10 minutes; blood: 3000 g, 10 minutes). The sera and saliva supernatant were stored at -20 Celsius degree (°C) till analyzed [19].

Biochemical biomarkers detection

Saliva and serum MDA were determined by spectrophotometry by using a thiobarbituric acid solution [20].

To measure the TAC, the colorimetric assay described by Erel [21] was used. The principle of this assay was depended on measuring the capacity to neutralize 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonate cationic root ($ABTS^+$) under the effect of the anti-oxidants that present in the same specimen. Absorbance of the $ABTS^+$ compound was detected in both serum and saliva specimens at 660 nanometer (nm).

The Pc content was detected and calculated by the assay of Levine *et al*. [22]. The introduction of the carbonyl group into the amino acid residues of proteins was considered the characteristic mechanism of oxidative modification by free radicals. The reaction of the carbonyl group with 2,4-dinitrophenylhydrazine forms a yellow colored 2, 4-dinitrophenylhydrazone which is spectrophotometrically measured. The absorbance for calculating the carbonyl content was estimated at 370 nm, by using the absorption coefficient (e) of 22,000 m^{-1} cm^{-1} and the Pc components was reported as nanomoles (nmol)/milligram (mg) protein [13].

The protein concentration in both serum and saliva was determined by the Lowry method, with used serum albumin of bovine as the standard for samples [13, 23].

The NO level in serum and saliva was measured by the assay of Cortas and Wakid (1990) in which nitrate was converted into nitrite using copper coated cadmium pellets. Thus, resulting nitrite was detected by linking sulfanilamide with naphthy-lethylenediamine to form a purple-colored compound. Color intensity was read at 540 nm with a spectrophotometer [24].

Menden method was used to measure Crp concentration in serum [25], and in saliva by Sunderman and Nomoto [26]. It depends on stimulating the oxidation of Crp from colorless paraphenin diamine (PPD) to an oxidized blue-violet compound. The reaction included photometry, determination of the blank value after enzyme inhibition by sodium azide, and reading of the results against the blank solution at 525 nm. The absorbance was directly related to Crp concentration.

$$\underset{\text{(Colorless)}}{\text{Reduced PPD} + 2H^+ + 1/_2O_2} \longrightarrow \underset{\text{(Blue violet)}}{\text{Oxidized PPD} + H_2O}$$

Statistical analysis

The Statistical Package for the Social Sciences (Version-24) was used. Descriptive statistics were included: means and standard deviations. The Chi square statistical method was used to test for differences between study groups. When the P-value was less than 0.05, the results were reported statistically significant.

Results and Discussion

Pregnant and breast-feeding women are at greater risk for oral and dental problems. The occurrence of some conditions such as hormonal disturbances, changes in oral cavity pH, and changes in dietary habits increased the incidence of dental caries and periodontal problems, including: gingivitis and periodontitis, [7]. During pregnancy, the gums swell and bleed more easily in most women due to the increased inflammatory response to dental plaque, and gingivitis may peak during the third trimester. Therefore, women with gingivitis before pregnancy are more likely to develop it during pregnancy [27].

One hundred (100) subjects [pregnant gingivitis patients (n=35), non-pregnant gingivitis subjects (n=35) and apparently healthy controls (n=30)] were enrolled in the current study. The age of study subjects was range from 19 to 42 years.

In this study, MDA concentration was measured to determine of the OS level. The MDA concentration within both gingivitis patients groups (pregnant and non-pregnant women) and their comparison with the healthy control group were expressed in Tables 1 and 2. The results revealed that the MDA concentration was significantly higher ($P^{a1,2}$=0.001) among pregnant gingivitis patients compared with healthy control group in both serum (3.55±0.67 nmol/ml vs. 1.31±0.15 nmol/ml) and saliva (1.52±0.13 nmol/ml vs. 0.33±0.12 nmol/ml). Thus, the presence of increased MDA levels indicated higher levels of OS, as a result of the increased OS during pregnancy [7]. The MDA concentration in non-pregnant gingivitis patients was also significantly higher ($P^{b1,2}$=0.001) compared with healthy controls in both serum (3.39±0.16 nmol/ml) and saliva (0.80±0.09 nmol/ml). Likewise, there was a significant different (P^{c1}=0.04) in serum MDA in pregnant gingivitis patients group (3.55±0.67 nmol/ml) compared with non-pregnant gingivitis patients group (3.39±0.16 nmol/ml). It is hypothesized that changes in the OS biomarkers in non-pregnant gingivitis patients are related to the inflammatory processes in gingivitis and directly with a bacterial load, meaning that any elevation in MDA levels of peroxide products will be correlated with several acute and chronic pathophysiological conditions [7]. The results indicated the validity of the MDA assay as a reliable tool in detecting OS in pathologies of various diseases [28]. In the Table 2, a higher MDA level (P^{c2}=0.01) was observed in the saliva of pregnant women with gingivitis (1.52±0.13 nmol/ml) compared with non-pregnant gingivitis patients women (0.80±0.09 nmol/ml). Saliva had important roles in protecting the oral cavity and oral health from microbial taxa and due to oral environmental changes during pregnancy, indicating that oral health is negatively affected by pregnancy due to abnormal growth of bacteria with increased OS in saliva [5].

Antioxidants are found in all tissues and body fluids. They protect cells from endogenously generated free radicals, which usually consist of electron transport system leakage [29]. In the same previous Tables, the TAC levels were significantly (P^{a1}=0.001, P^{a2}=0.01, $P^{b1,2}$=0.001) decreased in pregnant gingivitis patients and non-pregnant gingivitis patients compared to the control level in both serum (0.34±0.10 micromole (µmol)/ml and 0.41±0.09 µmol/ml, respectively vs. 0.66±0.13 µmol/ml) and saliva (0.51±0.13 µmol/ml and 0.49±0.15 µmol/ml, respectively vs. 0.73±0.22 µmol/ml). The total antioxidant capacity, is an important biomarker for measuring antioxidant potential in all types of body fluids, this antioxidant promotes periodontal health by providing protection against ROS-induced damage to periodontal tissues, as ROS levels rise pathologically, the antioxidants begin to work and help reduce oxidative damage and may be repaired or prevented, which can lead to low TAC [29]. One study found that a decrease serum and gingival crevicular fluid TAC level is correlated with pregnancy, especially in the latest 3 months, and thus, a decrease in TAC appears with deteriorating gingival to periodontal status in pregnant women [30], consistent with these findings, our study showed a significant (P^{c1}=0.01) depleted level of serum TAC among pregnancy gingivitis patients (0.34±0.10 µmol/ml) compared with non-pregnant gingivitis patients (0.41±0.09 µmol/ml). In addition, no statistically significant changes (P^{c2}=0.06) were recorded in the TAC level in the saliva of both pregnant and non-pregnant

women with gingivitis. The total antioxidant capacity associated with periodontal status can be affected by several constituents such as sex, pregnancy status, systematic conditions, and smoking [31]. However, it is not clear whether lower TAC levels and higher OS levels in saliva constitute an appropriate state of the bacterial environment in pregnant women due to pregnancy or whether bacterial growth helps reduce TAC and OS. Therefore, lower TAC levels indicate increased OS with potential for increased oxidative damage to gingival tissue and its progression to periodontal status [5], and thus, the antioxidant status of saliva correlates with both oral health and gum condition [11].

Oxidative stress and gingivitis severity are closely related. Recently, carbonyl compounds have acted as necessary indicators of OS due to their early formation and stability properties [32]. Furthermore, previous studies indicate the relationship between Pc, OS, and pathological conditions. Therefore, the formation of Pc causes damage and may degrade the function of the proteins [32]. According to the current study findings, the concentration of Pc was significantly increased in pregnant ($P^{a1,2}$=0.001) and non-pregnant (P^{b1}=0.01, P^{b2}=0.001) of gingivitis groups compared to healthy control group in both serum (2.71±0.91 nmole/mg and 1.90±0.81 nmole/mg, respectively vs. 1.34±0.53 nmole/mg) and saliva (1.22±0.40 nmole/mg and 1.51±0.29 nmole/mg, respectively vs. 0.77±0.34 nmole/mg), and this can be seen within the previous Tables (1 and 2). The presence of higher Pc levels indicates OS, and thus several factors had a vital role in determining the degree of protein damage and alteration [33]. Moreover, in many inflammatory immune diseases where OS plays an important role, including: gingivitis, chronic, and aggressive periodontitis, and therefore causes various changes in the properties of the periodontal protein, depending on the immune inflammation [32]. Moreover, in this study, after examining the serum and saliva of subjects with gingivitis, significantly ($P^{c1,2}$=0.01) higher Pc levels were observed in gingivitis patients during pregnancy (2.71±0.91 nmole/mg and 1.22±0.40 nmole/mg, respectively) compared with non-pregnant gingivitis patients (1.90±0.81 nmole/mg and 1.51±0.29 nmole/mg, respectively), suggesting that different mechanisms of active protein oxidation may be concomitant pregnancy. Other researchers had reported that OS may result from pregnancy itself due to increased metabolic activity in placental mitochondria and decreased scavenging power of antioxidants, confirming that the severity of gingivitis and periodontitis is directly related to the OS of pregnancy. It is for this cause that the high concentration of Pc in saliva with the category of patients with periodontitis leads to severe tissue destruction, since the high concentration of lipid peroxidation and protein products in non-stimulated saliva, generally reflects its plasma content. This confirms that biomolecules can be transported into the oral cavity by passive transport (proliferative and ultrafiltration), polycytosis, facilitated, and active transportation, so that the level of various biological molecules in saliva can be more concentrated than found in serum and/or plasma. Therefore, saliva is a highly gravitational fluid in the body for the diagnosis of differences conditions [34]. Furthermore, due to the correlation of biomarkers of salivary OS and its level in serum, the use of salivary oxidative markers in the oral cavity diseases detecting as well as systemic diseases can be frequently highlighted [35].

Oxidative stress is participated in the destruction of the gums when periodontitis occurs. Reactive oxygen species are involved in gum destruction when gingivitis occurs. An imbalance in oxidative activity may be a major factor in this inflammation, and the elevated TP level may provide a protective base against ROS [14]. Recent paper findings were indicated in Table 1, the highest concentration of TP in serum was in pregnant (9.11±0.99 gram (g)/deciliter (dl)) and non-pregnant women (8.60±1.33 g/dl) groups with gingivitis compared to healthy controls (5.91±0.87 g/dl) (P^{a1}=0.001 and P^{b1}=0.01, respectively). In the same Table, the results revealed a significant elevation (P^{c1}=0.01) in the level of serum TP in pregnant gingivitis patients (9.11±0.99 g/dl) compared with non-pregnant gingivitis patients (8.60±1.33 g/dl). Thus, the

elevated protein levels are likely due to contributions of acute phase interacting proteins, the concentration of which increases in serum during infection [14]. A significant (P^{a2}=0.001 and P^{b2}=0.01) higher level of protein in saliva was further observed in pregnant (0.56±0.11 g/dl) and non-pregnant women (0.51±0.18 g/dl) groups with gingivitis compared with healthy controls (0.25±0.09 g/dl) as in Table 2, and concentrations of salivary protein have been identified as biomarker of plasma protein leakage, which occurs as a result of the inflammatory process [1]. According to the findings of former table, non-significant change (P^{c2}=0.07) in the level of TP concentration in saliva was observed between both groups of pregnant (0.56±0.11 g/dl) and non-pregnant (0.51±0.18 g/dl) women with gingivitis. As confirmed by previous studies, when inflammation occurred, a number of changes were observed in the composition of salivary protein [1]. The elevated protein levels are likely due to protein contributions to the glandular saliva and flat fluid proteins. Thus, it has revealed a significant rise in gingivitis subjects, and may provide a pathway to improve oral healthcare [14].

Tables (1 and 2) depict NO levels in serum and saliva. Nitric oxide levels in both serum and saliva were statistically elevated ($P^{a1+a2+b1}$=0.001 and P^{b2}=0.01) in pregnant (66.82±9.3 µmol/liter (L) and 55.33±9.1 µmol/L, respectively) and non-pregnant (53.03±2.5 µmol/L and 49.93±9.5 µmol/L, respectively) women with gingivitis compared with the control group (31.64±4.4 µmol/L and 23.50±2.2 µmol/L, respectively). It has also been confirmed by reports of increased levels of nitrogen oxides in the blood in a range of diseases [24]. The most important catalyst for the NO synthesis is bacterial products, macrophages, and other inflammatory cells can stimulate their formation and release. Nitric oxide, which is produced by activated macrophages and in large quantities, is a cytotoxic compound that affects the capacity of the cells to kill microorganisms and cancer cells, furthermore to its effect on cellular proteins, deoxyribonucleic acid, and lipids. Thus, all of this leads to gingivitis [36]. Serum NO levels were further significantly increased (P^{c1}=0.001) for the group of pregnant women with gingivitis (66.82±9.3 µmol/L) compared with the group of non-pregnant women (53.03±2.5 µmol/L) with gingivitis. It has been observed that the increase in NO production through induced cytokine induction (nitric oxide-induced) is caused by pregnancy-related inflammation [37]. In the previous Table 2, significantly higher levels of salivary NO (P^{c2}=0.01) can be reported in the case of the pregnant women group (55.33±9.1 µmol/L) compared with the non-pregnant women group (49.93±9.5 µmol/L) with gingivitis, because the conditions of a woman's organs during pregnancy is subject to several changes such as hormonal, physiological, and neurological changes. In turn, the oral cavity reveals some cases of changes during this period, including: purulent granulomas, gingivitis, and periodontitis [37].

Table 1: A Comparison between biochemical biomarkers in serum of all study groups

Groups / Biomarkers (Serum)	MDA (nmol/ml)	TAC (µmol/ml)	Pc (nmole/mg)	Protein (g/dl)	NO (µmol/L)	Crp (mg/dl)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Pregnant Gingivitis Patients (n=35)	3.55±0.67	0.34 ±0.10	2.71 ±0.91	9.11±0.99	66.82± 9.3	53.1±2.2
Non-pregnant Gingivitis Patients (n=35)	3.39±0.16	0.41±0.09	1.90±0.81	8.60±1.33	53.03 ±2.5	44.24±6.2
Healthy Controls (n=30)	1.31±0.15	0.66 ±0.13	1.34±0.53	5.91±0.87	31.64 ±4.4	27±4.3
P-value	0.001^{a1} 0.001^{b1} 0.04^{c1}	0.001^{a1} 0.001^{b1} 0.01^{c1}	0.001^{a1} 0.01^{b1} 0.01^{c1}	0.001^{a1} 0.01^{b1} 0.01^{c1}	0.001^{a1} 0.001^{b1} 0.001^{c1}	0.001^{a1} 0.001^{b1} 0.01^{c1}

n: *number,* ***MDA:*** *malondialdehyde,* ***nmol:*** *nanomoles,* ***ml:*** *milliliter,* ***SD:*** *standard deviation,* ***TAC:*** *total antioxidant capacity,* ***µmol:*** *micromole,* ***Pc:*** *protein carbonyl,* ***mg:*** *milligram,* ***g:*** *gram,* ***dl:*** *deciliter,* ***NO:*** *nitric oxide,* ***L:*** *liter,* ***Crp:*** *ceruloplasmin,* ***a1:*** *a comparison between pregnant gingivitis patients and healthy control,* ***b1:*** *a comparison between non-pregnant gingivitis patients and healthy control and* ***c1:*** *a comparison between pregnant gingivitis patients and non-pregnant gingivitis patients.*

Table 2: A Comparison between biochemical biomarkers in saliva of all study groups

Groups/ Biomarkers (Saliva)	MDA (nmol/ml)	TAC (µmol/ml)	Pc (nmole/mg)	Protein (g/dl)	NO (µmol/L)	Crp (mg/dl)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Pregnant Gingivitis Patients (n=35)	1.52±0.13	0.51±0.13	1.22±0.40	0.56±0.11	55.33 ± 9.1	0.89±0.33
Non-pregnant Gingivitis Patients (n=35)	0.80±0.09	0.49±0.15	1.51±0.29	0.51±0.18	49.93 ± 9.5	0.87±0.29
Healthy Controls (n=30)	0.33±0.12	0.73±0.22	0.77±0.34	0.25±0.09	23.50 ± 2.2	0.57±0.21
P-value	0.001^{a2} 0.001^{b2} 0.01^{c2}	0.01^{a2} 0.001^{b2} 0.06^{c2}	0.001^{a2} 0.001^{b2} 0.01^{c2}	0.001^{a2} 0.01^{b2} 0.07^{c2}	0.001^{a2} 0.01^{b2} 0.01^{c2}	0.001^{a2} 0.001^{b2} 0.06^{c2}

n: *number,* ***MDA:*** *malondialdehyde,* ***nmol:*** *nanomoles,* ***ml:*** *milliliter,* ***SD:*** *standard deviation,* ***TAC:*** *total antioxidant capacity,* ***µmol:*** *micromole,* ***Pc:*** *protein carbonyl,* ***mg:*** *milligram,* ***g:*** *gram,* ***dl:*** *deciliter,* ***NO:*** *nitric oxide,* ***L:*** *liter,* ***Crp:*** *ceruloplasmin,* ***a2:*** *a comparison between pregnant gingivitis patients and healthy control,* ***b2:*** *a comparison between non-pregnant gingivitis patients and healthy control and* ***c2:*** *a comparison between pregnant gingivitis patients and non-pregnant gingivitis patients.*

One of the biomarkers that can reveal its level change in many inflammatory conditions such as gingivitis and periodontitis is Crp, with which periodontal risk can be determined and quantified [18]. The findings of the current research revealed that the Crp levels were significantly (P^{a1+b1}=0.001 and P^{a2+b2}=0.001) increased in gingivitis patients during pregnancy (53.1±2.2 mg/dl and 0.89±0.33 mg/dl) and non-pregnant gingivitis patients (44.24±6.2 mg/dl and 0.87±0.29 mg/dl) compared with their levels in healthy control (27±4.3 mg/dl and 0.57±0.21 mg/dl) in both serum and saliva, respectively as indicated in Tables (1 and 2). The localized inflammatory state is characterized by decreased tissue oxygen content, by generation of oxygen free radicals in polymorphous neutrophils, neutrophil-mediated tissue injury, and increased OS, the fact may be that it is involved in tissue damage during the period of inflammatory conditions, and this may be due to the fact that the Crp level appears to be significantly elevated only in the presence of active tissue destruction [18, 38]. This could be the reason for the elevated Crp levels in this study. Through Table 1, it was found that the Crp concentration in the serum of gingivitis patients was significantly higher (P^{c1}=0.01) during pregnancy (53.1±2.2 mg/dl) compared with non-pregnant women (44.24±6.2 mg/dl) with gingivitis, and this confirms that the Crp levels in the serum were found to increase during normal pregnancy. This Crp increase during pregnancy is thought to protect against high levels of OS associated with pregnancy, as well as the presence of gingivitis during pregnancy [39]. Salivary concentration of Crp also showed non-significant (P^{c2}=0.06) increase in pregnant compared with non-pregnant women with gingivitis as depicted in the previous Table 2. Therefore, the salivary findings could be interpreted on the basis that several molecules including Crp can penetrate the gingival tissues through the intercellular spaces of the junction epithelium to saliva, and this finding could reflect the severity of the disease [40]. Therefore Crp can be used as a diagnostic assay to evaluate gingivitis and periodontal disease activity.

The major limitations of our study was small sample size because only patients that admitted to Dental Center in Thi-Qar province were included and the subjects selection criteria (exclusion and inclusion) had the major effect to decrease sample size of the current study, because of there were a some difficulty to find a subject that met it. The second limitation was the actual sensitivity and specificity of the techniques that used for biomarkers measuring in both serum and saliva samples. In addition, some women continued to preserve their pregnancy using certain gynecology medications, it is impossible to rule out the possibility that these drugs affected the OS status of them. Further studies are needed to

identify the precise mechanisms underlying the changes in the OS status in pregnant women with gingivitis.

Conclusion

According to the findings of current study, we concluded that the pregnancy status had an effect on the OS levels which lead to elevated concentrations of OS in both serum and/or saliva of gingivitis patients and the correlation between the pregnancy status and serum and/or saliva OS levels was positive which indicated the possible utility of its present lead to gingivitis development. There are urgent needs for further future studies with a large sample size to clarify the role of other OS biomarkers in the pathogenicity of gingivitis among pregnant women.

Acknowledgment

We would like to extend our thanks to the staff of the Dental Center in Thi-Qar Province for their unlimited assistance and efforts during the collection of samples, and a special thanks to the staff of the Community Health Department/Al-Nasiriyah Technical Institute for their assistance in completing the practical side in the clinical biochemistry laboratory by measuring the biochemical tests of samples. We also expressed our full heart gratitude to all the participants for their greater assistance and cooperation.

Funding

This research did not receive any specific grant from fundig agencies in the public, commercial, or not-for-profit sectors.

Author's Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

There are no conflicts of interest in this study.

List of Abbreviates

µmol: micromole, **ABTS:** 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonate, **Crp:** ceruloplasmin, **ºC:** degree celsius, **dl:** deciliter, **g:** gram, **hrs:** hours, **L:** liter, **MDA:** malondialdehyde, **mg:** milligram, **ml:** milliliter, **mM:** millimolar, **nm:** nanometer, **nmol:** nanomoles, **NO:** nitric oxide, **OS:** oxidative stress, **Pc:** protein carbonyl, **PPD:** paraphenin diamin, **ROS:** reactive oxygen species, **TAC:** total antioxidant capacity and **TP:** total proteins.

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HOW TO CITE THIS ARTICLE
Wisam R. N. Al-Muhsen, Hasan Abd Ali Khudhair, Nisreen Ahmed Hamzah. Study the Effect of Pregnancy on Oxidative Stress Status in Pregnant Women with Gingivitis. *J. Med. Chem. Sci.*, 2022, 5(7)1253-1264
https://doi.org/10.26655/JMCHEMSCI.2022.7.15
URL: http://www.jmchemsci.com/article_153763.html