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The Study of Some Antioxidant Markers, Malondialdehyde and C - reactive protein in Suckling Women

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ABSTRACT

This study aims to find out the role of some antioxidants markers (Glutathione GSH and superoxide dismutase SOD) , malondialdehyde (MDA) and C-reactive protein (CRP) in suckling women .The whole sample is about 120 women (aged 25-35 years) , included 60 suckling women and 60 non-suckling . Each of these both suckling and non - suckling women divided into three groups (20 women / group) according to the periods of suckling as the following : First group (six months) , Second group (twelve months) , Third group (eighteen months) . Results revealed : GSH and SOD levels decreased significantly ($p \leq 0.05$) in non - suckling women in comparison with the suckling women for different groups . MDA levels increased significantly($p \leq 0.05$) in non - suckling women in comparison with the suckling women for different groups . CRP increased significantly ($p \leq 0.05$) in non-suckling women in comparison with the suckling women for different groups . The physiological impacts for these changes be discussed according to the role of suckling and their positive effects on those women related with these current parameters .

Key words: Glutathione, Superoxide dismutase, Malondialdehyde , C- Reactive protein .

INTRODUCTION

An imbalance between reactive oxygen species (ROS) and antioxidant defenses is therefore a pathological condition that not only leads to direct cellular damage but also leads to an inflammatory cascade that increases the perpetuates tissue injury (Charlton et al ., 2021) , it is a harmful process that can negatively affect several cellular structures , such as membranes, lipids , proteins, lipoproteins , and deoxyribonucleic acid (DNA) (Droge , 2001 ;Young and Woodside 2001; Pizzino et al ., 2017) , in addition , oxidative stress induces the activation of pro-inflammatory cytokines and subsequent inflammation which further promotes the production of ROS , thereby damaging cells and tissues (Oguntibeju , 2019 ; Charlton et al .,2021).

Endogenous enzymatic and nonenzymatic antioxidants are a part of the body's complex antioxidant protection grid . These molecules work together to combat free radicals and their adverse effects on essential biomolecules and , finally, body tissues (Ighodaro and Akinloye , 2018) . They can be classified as first, second, third, or even fourth line defense antioxidants based on their response to general free radical invasion , The first line protection antioxidants, which primarily include superoxide dismutase

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(SOD), catalase (CAT), and glutathione peroxidase (GPX), play a significant and indispensable role in the overall antioxidant defense strategy, especially in relation to superoxide anion radical (*O2) which is constantly produced in normal body metabolism, particularly via the mitochondrial energy production pathway (MEPP) (Ighodaro and Akinloye , 2018).

Lactation is the most energetically demanding time of a female's life in mammals, and it is marked by a dramatic increase in the organism's energy and nutrient needs for milk production (Gutgesell et al ., 2009; Pichaud et al . , 2013) , In order to satisfy the increased energy demands of offspring growth and somatic defense, both energy intake and expenditure are increased (zheng et al., 2015). Many researchers believe that oxidative stress is a physiological expense of reproduction that has the potential to affect future female reproductive success and longevity (Monaghan et al., 2009 ; Dowling and Simmons , 2009) . Despite the fact that this theory has gained popularity, the findings of studies that have tested its validity have yielded mixed results (Speakman and Garratt , 2014).

Some physiological reproductive events such as ovulation, menstruation , implantation and the initiation of labor show distinct signs of inflammation , which are regulated by specific molecular pathways that include a range of growth factors , cytokines, chemokines , and lipid mediators (Jabbour et al ., 2009). After giving birth (4 to 6 weeks) postpartum women , particularly those who are exclusively breastfeeding , have heightened and triggered innate and unique immune defenses (Groer et al., 2005) , however , study indicated the lack of evidence for pro-inflammatory changes during lactation (Kuzawa et al., 2013).

González-Fernández and his colleagues (2017) found that infection both raise and lower CRP concentrations in pregnant and lactating mothers , and also , CRP increased in mothers , during lactation folic acid deficiency as associated with higher CRP concentrations .Sproston and Ashworth (2018) suggested that CRP is not just a marker of inflammation or infection but an important regulator of inflammatory processes .

In view of fore going , this study is an attempt to shed some light about the antioxidants markers (GSH and SOD) , MDA and CRP in suckling women .

MATERIALS AND METHODS

The sample of this study included (120 women) aged 25-35 years , included sixty suckling women and sixty non-suckling women . Each these both suckling and non-suckling women divided into three groups (20 women / group) according to the periods of suckling as the following : First group (six months) , Second group (twelve women) , Third group (eighteen months) .

The blood samples prepared by the usual procedure in order to measure glutathione, superoxide dismutase , MDA and C- reactive protein . Glutathione (GSH) was performed using Glutathione (GSH) enzyme immunoassay kits (Elabscience / USA) , according to the manufacturer’s instructions . Sandwich enzyme-linked immune-sorbent assay technology for the quantitative determination of Superoxide dismutase [Cu-Zn](SOD1) was performed using Human SOD1 (Superoxide dismutase [Cu-Zn])

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enzyme immuno assay kits (Elabscience / USA), according to the manufacturer's instructions. Enzyme linked immunosorbent assay (ELISA) system for the quantitative determination of Malondialdehyde (MDA) was performed using Human MDA enzyme immunoassay kits (Elabscience / USA), according to the manufacturer's instructions.C-reactive protein (CRP) was measured usingparticle enhanced turbidimetric assay Human CRP agglutinates with latex particles coated with monoclonal anti- CRP antibodies (Roch / Germany) , according to the manufacturer's instructions .The results are expressed as Mean ± Standard Division (SD) , Statistical analysis was performed by IBM SPSS statistics , version 26 (IBM Co., Armonk , NY, USA) . The statistical analysis was performed by one-way Analysis Of Variance (ANOVA) , followed by a t-test for the different groups .

RESULTS

Oxidant and antioxidant markers

Glutathione (GSH) :

First Group

Results revealed : GSH levels in the non - suckling women(6.945 ±0.925 μg/ml) decreased significantly ($p \leq 0.05$) in comparison with the suckling women (8.585 ±0. 950 μg/ml) . Figure (1) .

Second Group

Results revealed : GSH levels in the non - suckling women (5.795 ±0.933 μg/ml) decreased significantly ($p \leq 0.05$) in comparison with the suckling women (7.050 ±0.623 μg/ml) . Figure (1) .

Third Group

Results revealed : GSH levels in the non - suckling women (5.065 ±0.940 μg/ml) decreased significantly ($p \leq 0.05$)in comparison with the suckling women (6.940 ±0.534 μg/ml) . Figure (1)

Superoxide Dismutase (SOD) :

First Group

Results revealed : SOD levels in the non - suckling women (6.165 ±0.914 ng/ml) decreased significantly ($p \leq 0.05$) in comparison with the suckling women (8.360 ±0.926 ng/ml) . Figure (2) .

Second Group

Results revealed : SOD levels in the non - suckling women (6.906 ±0.899 ng/ml) decreased significantly ($p \leq 0.05$) in comparison with the suckling women (9.035 ±0.692 ng/ml) . Figure (2) .

Third Group

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Results revealed : SOD levels in the non - suckling women (8.720 ±0.905 ng/ml) decreased significantly ($p \leq 0.05$) in comparison with the suckling women (9.948 ±0.888 ng/ml) . Figure (2) .

Malondialdehyde (MDA) :

First Group

Results revealed : Malondialdehyde (MAD)levels in the non - suckling women (122.672 ±16.779 ng/ml)increased significantly ($p \leq 0.05$) in comparison with the suckling women (90.435 ±8.776 ng/ml) . Figure (3) .

Second Group

Results revealed : MAD levels in the non - suckling women (226.040 ±14.983 ng/ml) increased significantly ($p \leq 0.05$) in comparison with the suckling women (88.910 ±14.825 ng/ml) . Figure (3)

Third Group

Results revealed : MAD levels in the non - suckling women (227.745 ±16.405 ng/ml) increased significantly ($p \leq 0.05$) in comparison with the suckling women (76.030 ±9.802 ng/ml) . Figure (3) .

C-reactive protein (CRP) :

First Group

Results revealed : (CRP) levels in the non - suckling women (1.690±0.554 mg/dl)) increased significantly ($p \leq 0.05$) incomparison with suckling women (0.716±0.202 mg/dl) . Figure (4) .

Second Group

Results revealed : (CRP) levels in the non - suckling women (1.209 ±0.387mg/dl) increased significantly ($p \leq 0.05$) in comparison with the suckling women (0.703±0.170mg/dl) . Figure (4) .

Third Group

Results revealed : CRP levels in the non - suckling women (0.723±0.250mg/dl) increased significantly ($p \leq 0.05$)in comparison with suckling women (0.691±0.186mg/dl) .Figure (4) .

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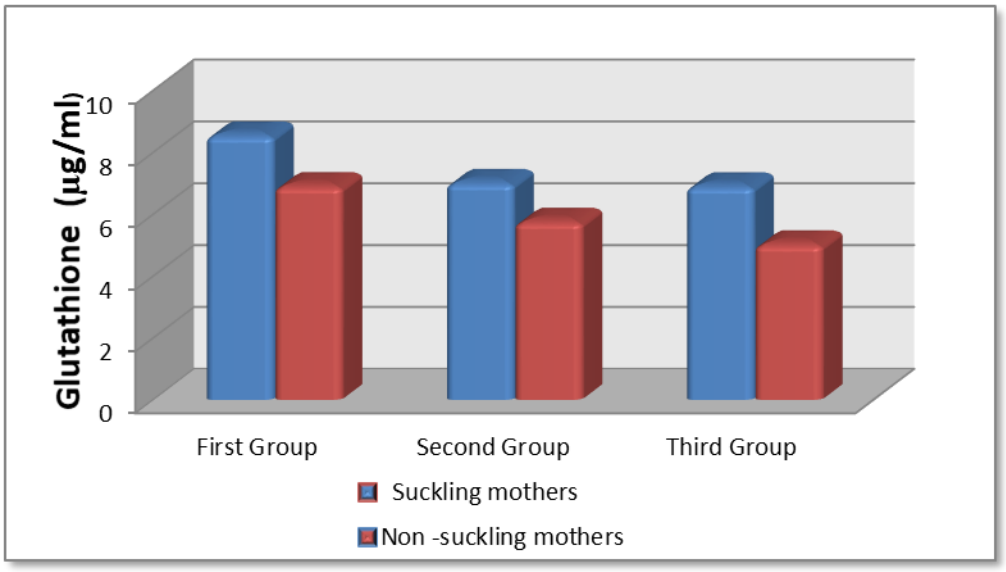

Figure (1) : Glutathione levels in suckling and non - suckling mothers for different groups .

The values represent mean ± SD .

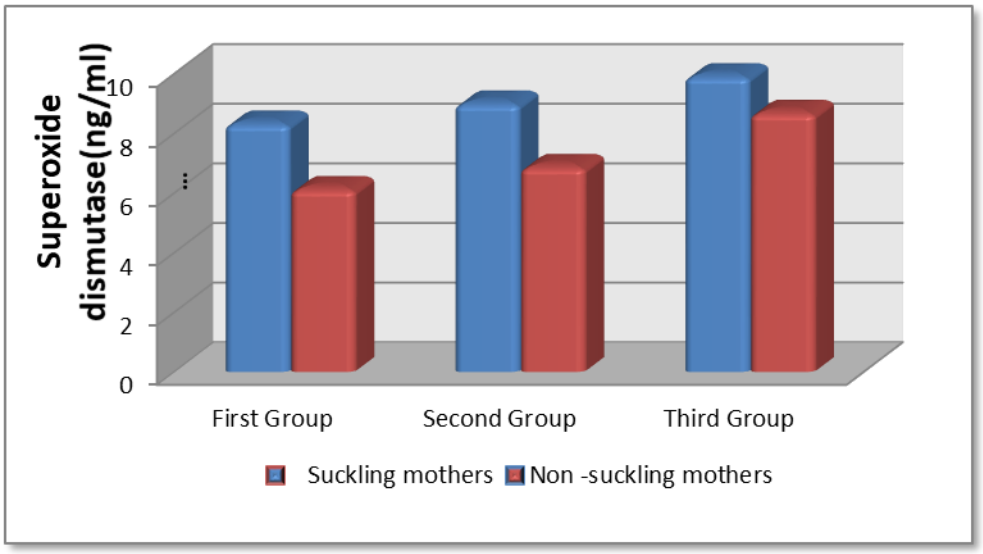

Figure (2): Superoxidedismutase levels in suckling and non - suckling mothers for different groups .

The values represent mean ± SD .

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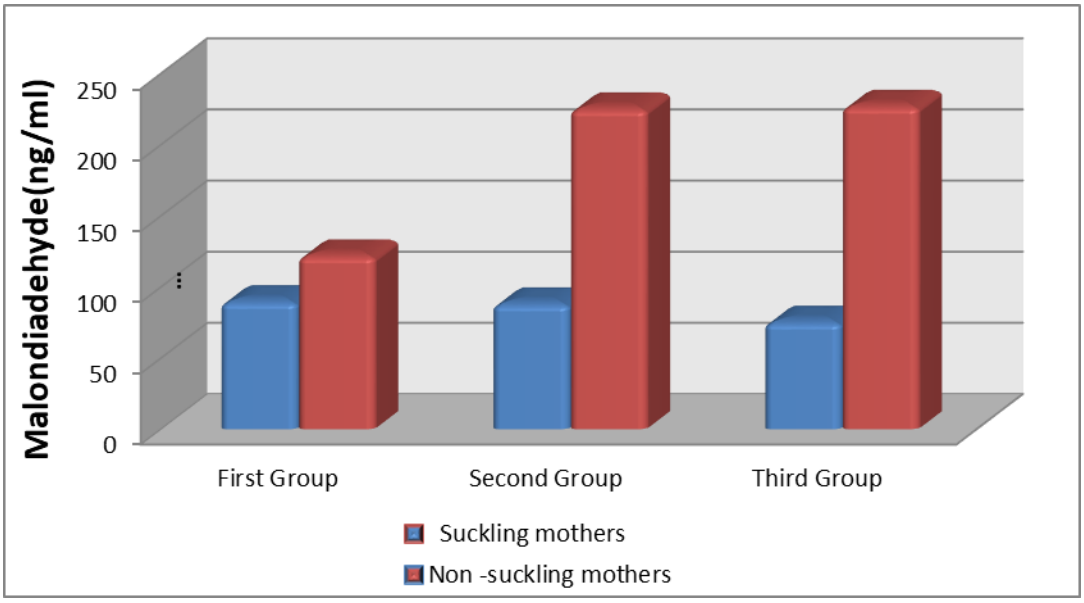

Figure (3): Malondialdehyde levels in suckling and non - suckling mothers for different groups .

The values represent mean ± SD .

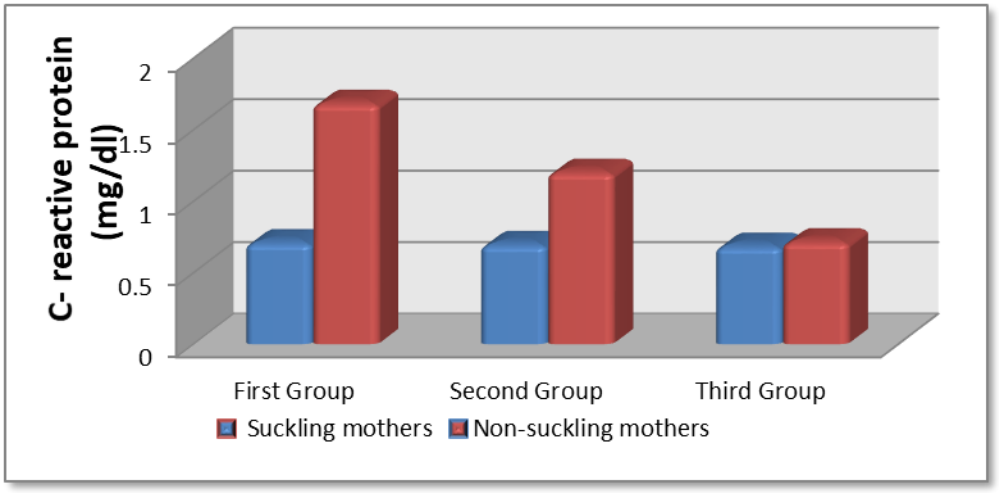

Figure (4): C- reactive protien levels in suckling and non - suckling mothers for different groups .

The values represent mean ± SD .

DISCUSSION

The present results revealed that the antioxidants glutathione (GSH) and superoxide dismutase (SOD) levels decreased significantly for non – suckling women in comparison with the suckling women for different groups . Figure (1) and Figure (2) , while , malondialdehyde(MDA) levels increased significantly in non - suckling women in comparison with the suckling women for

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different groups . Figure (3) .The present reduction in GSH and SOD antioxidants with high MDA level in non - suckling women , may be attributed to the overproduction of reactive oxygen species (ROS) and decreased production of antioxidants (SOD , GSH) . In other words , the high production of reactive oxygen species (ROS) overtakes the women 's antioxidant capacity due to the increase of oxidation processes that occurred via the accumulation of storage , un packaged and un used fats in the lactation process or their metabolites . The present results concerning the high levels of MDA in non- suckling women attributed to high oxidation and oxidative stress , thus , more production of ROS species that be harmed and damaged to cells and their functions , whereas , the reduction of MDA levels in suckling women , reflects high activity of antioxidant system , while , the current study results pointed out high activities of antioxidants in suckling women .

Regarding the oxidative stress and the antioxidant defense system of breast-feeding mothers , this oxidative stress level was highest and the antioxidant power level was lowest in the early puerperium , the antioxidant power level then showed a clear tendency to recover and the oxidative stress gradually decreases in the first 3 months after giving birth , moreover , the antioxidant potential of breast milk is higher in mature milk than in colostrum or transitional milk (Kuramoto and Kitagawa , 2017) .

Zheng and his colleagues (2015) found that antioxidant activities regulated physiologically in response to elevated ROS production , during the peak of lactation , and several markers of oxidative stress did not increase in lactating mice compared with that in non-reproductive counterparts , MDA levels were significantly lower in liver , kidneys , skeletal muscle and small intestine in lactating females than non-reproductive controls, indicating that no evidence of increased oxidative stress was found in reproductive mice .

Valencak and his colleagues (2016) suggested that metabolism in reproducing females mice is well prepared for the challenges of lactation and either fights back , tolerates or even decreases ROS production , this contribute to growing evidence that there is no linear relationship between oxidative damage and lactation .

Hyatt and his colleagues (2018) reported that rats who experienced lactation had select markers of oxidative stress after lactation had ended and mammary tissue had regressed . Where a higher level of SOD2 was found in the liver and in WAT , In addition , no statistical differences were

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detected for SOD1, GPX levels in liver and also SOD2 , SOD1 , CAT, GPX levels in skeletal muscle , when , comparing with non- suckling rats and non reproduce rats .

Xu and his colleagues (2014) found that lactating mice had significantly lower protein carbonyls (measured by MDA) in their livers compared with non-reproductive voles , indicating oxidative damage in the liver was decreased during lactation , in addition , a significant increase in SOD levels activity in the livers of lactating mice , and this may explain the reason for the decrease in oxidative damage in the liver.

In study of Garratt and his colleagues (2011) in reproductive mice liver , markers of oxidative damage (malonaldehyde , protein thiols and the proportion of glutathione in the oxidized form) indicated lower oxidative stress in reproducing females when compared with non-reproductive controls , even during peak lactation , none of the markers of oxidative damage indicated higher oxidative stress than among non-reproductive females .

Yang and his colleagues (2013) found that markers of oxidative damage MDA , protein carbonyls in the liver were lower , while increased levels of SOD activity and total antioxidant capacity in the liver in lactating compared with non-reproductive gerbils .

- Furthermore , several markers of oxidative stress did not increase in lactating mice compared with that in non-reproductive counterparts , MDA levels were significantly lower in liver , kidneys , skeletal muscle and small intestine in lactating females than non-reproductive controls, indicating that no evidence of increased oxidative stress was found in reproductive mice (Zheng et al . , 2015) .

Meanwhile , the present CRP increased significantly in non-suckling women in comparison with the suckling women for different groups . Figure (4).

This significant increase in CRP may be indicated a presence of an inflammation state in these non – suckling women , in according to the high levels of CRP which used as an indicator of inflammation and its stimulation by several cytokines including IL6 , beside that CRP responds to a variety of maternal factors including the present increase finding of oxidative stress associated with a high values of CRP in non – suckling women .

C-reactive protein (CRP) is a well-established marker of inflammation , low-grade inflammation (LGI), defined as slightly increased CRP levels, is associated with increased risk of several diseases (Dinh et al ., 2019).

Levels of CRP increase very rapidly in response to trauma , inflammation , and infection and decrease just as rapidly with the resolution of the condition , thus, the measurement of CRP is widely used to monitor various inflammatory states (Du Clos, 2000) .CRP is the best-known acute phase protein , in humans , almost every type of inflammation is accompanied by an increase of CRP concentration , until recently, the only known physiological function of CRP was the marking of

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cells to initiate their phagocytosis (Sheriff et al ., 2021) . CRP produced in large amounts by hepatocytes ,

upon stimulation by the cytokines interleukin-6 , tumor necrosis factor alpha and interleukin-1beta , during an acute-phase response (Vermeire et al ., 2005) .

Furthermore , oxidative stress may be a determinant of CRP levels and promote pro-atherosclerotic inflammatory processes at the earliest stages of coronary heart disease development (Abramson et al ., 2005).

Dohi and his colleagues (2007) indicated that C-reactive protein levels associated with oxidative stress, in addition to, where, found that CRP levels was positively correlated with 8-isoprostane (an oxidative strees marker) and triglycerides and inversely correlated with high-density lipoprotein –cholesterol . Otherwise , pregnancy and lactation involved in adaptations of immune regulation (Kurzua et al ., 2013) . The post-par turn period , regardless of feeding method , seems to be oriented towards heightened and activated innate and specific immune defenses , while breastfeeding provides an additional level of potential protection for these mothers and their infants (Groer et al ., 2005) . The immunological effects of pregnancy remains until about 1 year after delivery (Watanabe et al ., 1997) .

According to these oxidant , antioxidant and pro – inflammatory markers , we can concluded that the non – suckling women may be predispose some metabolic diseases in the future if they are not corrected .

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