

Oxidants -Antioxidant Status in Neonatal Jaundice with Severe G6PD Deficiency

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Abstract

Summary: Study of oxidative stress and some anti-oxidants with lipid peroxidation end product malondialdehyde in male neonatal jaundice with severe G6PD deficiency in Diwaniyah province of Iraq and the obtained results were compared with that found in control group. The mean \pm SD of antioxidant status and oxidative stress parameters which include erythrocyte GSH, MDA, G-Red, G-Px and catalase were determined. There was a significant decrease in each of erythrocyte GSH, G-Red and catalase activity levels ($P < 0.05$), whereas the lipid peroxidation end product MDA levels and G-Px activity levels were significantly increased in all neonatal jaundice ($P < 0.05$) as compared with the control group. G6PD activity values identified were found to be positively correlated with each of GSH concentrations, G-Red and catalase activity levels in which their values were found to decrease in patient groups, while it was found to be negatively correlated with each of G-Px activity and MDA levels in which their values were elevated in severe G6PD-deficient neonates. These data indicates an increases in free radical generation and thus antioxidant defense mechanisms is impaired in peroxidation associated with a significant elevation in MDA levels in the erythrocytes of neonatal jaundice with severe G6PD deficiency than that found in the control group which demonstrate the presence of an increased oxidative stress due to reduction in NADPH which is generated in RBCs by HMP-shunt only.

الخلاصة

تم دراسة حالة جهد التأكسد وبعض مضادات الأوكسدة وكذلك متابعة ناتج أكسدة الدهون المالونداي ألديهيد Malondialdehyde في الذكور حديثي الولادة والمصابين بمرض اليرقان الولادي بسبب النقص الحاد في نشاط إنزيم الـ G6PD في محافظة الديوانية ومن ثم مقارنة النتائج مع مجموعة السيطرة. وقد أظهرت الدراسة وجود زيادة معنوية في كل من تركيز الـ MDA ونشاط إنزيم الـ G-Px ($P < 0.05$)، بينما جاءت نتائج كل من الـ GSH, G-Red, Catalase على العكس حيث انخفضت معنويا عند مقارنتها مع مجموعة السيطرة ($P < 0.05$). ومن جانب آخر فقد وجد أن هناك ارتباطا معنويا سالبيا ما بين نشاط إنزيم الـ G6PD المنخفض بشكل حاد مع كل من نشاط إنزيم الـ G-Px وتركيز الـ MDA وتم إيجاد معامل الارتباط لكل حالة، بينما كانت العلاقة موجبة معنويا مع كل من تركيز الـ GSH ونشاط كل من إنزيمي الـ G-Red and Catalase وتم إيجاد معاملات الارتباط لكل منهما. وقد تعود أسباب هذه التغيرات إلى النقصان الحاد في نشاط إنزيم الـ G6PD وبالتالي قلة التخليق الحيوي للـ NADPH ومن ثم نقصان الـ GSH والإنزيمات ذات العلاقة وحدوث زيادة في توليد الجذور الحرة وكذلك بالنسبة لناتج أكسدة الدهون الـ MDA.

Introduction

A homeostasis between rate of free radicals generation and the rate of their

neutralization if not maintained, oxidative damage accumulates and is known as oxidative stress (Sies, 1991). Neonatal jaundice is a normal physiological event

that is being treated on a belief of pathology. Commonly neonatal jaundice occurs for two reasons:

1. Infants have too many red blood cells. It is a natural process for the baby's body to break down these excess red blood cells, forming a large amount of bilirubin. It is this bilirubin causes the skin to take an yellowish color.

2. A newborn's liver is immature and cannot process bilirubin as quickly as the baby will be able to gets older. This slow processing of bilirubin has nothing to do with liver disease. It merely means that the neonates liver is not as fully developed as it will be, and thus, there is some delay in eliminating the bilirubin. Neonatal jaundice affects 60% of full-term infants and 80% of preterm infants in the first 3 days after birth. Antioxidant activity in serum of term neonates is lower than that of adults and is still lower in preterm and low birth weight babies as compared to term babies (Sullivan and Newton, 1988). Red blood cells are extremely susceptible to lipid peroxidation since they are rich in unsaturated membrane lipids, have rich supply of oxygen and transitional metal catalyts. Neonatal erythrocyte membrane is more susceptible to oxidative damage due to its predominant pro-oxidant potential (Jain, 1989). The erythrocytes are particularly prone to the free radical damage since the membrane lipids are very rich in polyunsaturated fatty acids which play an essential role in generating free radicals. As free radicals are potentially toxic, they are usually inactivated or scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids. Alteration in the oxidant-antioxidant profile is known to occur in neonatal jaundice (Turgut, et. al., 2004). Moreover the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or

oppose their actions (Sies, 1991) and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated (Cotgreave, et. al., 1988). They exist in both the aqueous and membrane compartment of cells and can be enzymes or non-enzymes. The human body has a complex antioxidant defense system that includes the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (G-Px) and catalase (CAT). These block the initiation of free radical chain reactions (Mahadik and Soheffer, 1996) The non-enzymatic antioxidant components consists of various molecules such as glutathione (GSH), vitamin E, ascorbic acid and beta-carotene that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions In the present study, malondialdehyde (MDA) ; glutathione (GSH) ; and the antioxidant enzymes catalase, glutathione peroxidase (G-Px) and glutathione reductase (G-Red) were determined in erythrocyte of neonatal jaundice with severe G6PD deficiency and compared with that found in control group. This is the first attempt to examine oxidative stress and the status of the protective antioxidants under condition of stress due to the neonatal jaundice in Diwaniyah Province.

Materials and Methods

A total of 145 blood samples were collected from full-term male neonatal jaundice with TSB \geq 15 mg/dl and another 43 control neonates with age ranged between 1-28 days which were admitted in Diwaniyah Teaching Hospital of Pediatric and Maternity / Diwaniyah - Iraq during 1st , Oct., 2007 and 1st , Feb. , 2009 and they received phototherapy when their TSB levels exceed 15 mg/dl. All the neonates were being breastfed and had no etiological factor for jaundice. Any G6PD-deficient neonates with other possible

etiologies causing jaundice, such as infants of diabetic mothers, polycythemia, perinatal infection, gastrointestinal obstruction, prematurity, ABO incompatibility, birth asphyxia, sepsis or those that had received intensive phototherapy; those in which the TSB level rose by more than 5 mg/dl per day or was higher than 20 mg/dl within the first 24 hours after birth; and those with signs and symptoms suggestive of serious illness were excluded. Blood samples in a quantity of (2-3 ml) were taken from a peripheral vein in EDTA anticoagulant collecting tube (300 μ L EDTA , 0.5 M) from both full-term male control and neonatal jaundice which were centrifuged at 1500 rpm for 10 minutes within 20 minutes of collection. Serum samples were stored at -20°C and analyzed in duplicate for biochemical and oxidative stress parameters assays within two weeks. **G6PD** activity levels was measured quantitatively in hemolysates by using Sigma kit (345-B) based on kinetic method recommended by WHO in 1967 and was modified modified by (Kornberg, and Horecker, 1955). Their activity level was expressed in micromole of NADPH formed per minute per gram hemoglobin in hemolysates. Hemoglobin concentration was determined and the G6PD activity was expressed as international units per gram hemoglobin (U/g Hb) in erythrocyte hemolysate. Total and conjugated serum bilirubin in control and neonatal jaundice were determined according to a modified method (Doumas and Wu, 1991). The erythrocyte lipid peroxidation end product malondialdehyde (MDA) level was determined by a method depends upon the reaction with thiobarbituric acid (TBA) at $90-100^{\circ}\text{C}$ (Esterbauer and Cheeseman, 1990). The level of reduced glutathione (GSH) in erythrocytes was determined by Beutler method as a modification of the Ellman method (Beutler, et. al. 1963). Erythrocyte were deproteinated by addition of trichloroacetic acid (TCA). DTNB [5,5'-

dithio-bis-(2-nitrobenzoic acid)] was added to supernatants cleared by centrifugation (10 min, 3000 rpm). The formation of 5-thio-2-nitrobenzoic acid, which is proportional to total glutathione concentration, was monitored at 412 nm at 25°C against reagent controls. **G-Red** activity was measured by Randox G-Red assay kit provides an indirect and highly reproducible method of quantifying the G-Red activity in hemolysates which is an important measure of the antioxidant status of the cell. The oxidation of NADPH to NADP^{+} is accompanied by a decrease in absorbance at 340 nm (A_{340}), thus providing a spectrophotometric means of detection which is directly proportional to the G-Red activity in the sample (Goldberg and Spooner, 1983). **G-Px** activity was measured according to ZeptoMetrix diagnostic kit for G-Px activity measurements, USA. Cumene hydroperoxide is used as the peroxide substrate (ROOH), and glutathione reductase (GSSG-R) and β -NADPH are included in the reaction mixture. The formation of GSSG catalyzed by G-Px is coupled to the recycling of GSSG back to GSH using G-Red in which NADPH is oxidized to NADP^{+} . The rate of decrease in absorbance at 340 nm due to NADPH oxidation is monitored spectrophotometrically and is directly proportional to the total activity of G-Px. Since all other reagents are provided in excess, the amount of G-Px in the test sample is the rate-limiting factor. Cumene hydroperoxide is suitable for the reaction because it has a low spontaneous reaction with GSH, low spontaneous hydrolysis and is not metabolized by Catalase, another universally present antioxidant enzymes. G-Px activity both in plasma and in RBC hemolysate can be determined with this kit which can also be adapted to G-Px activity determination in cells from culture and tissue homogenates (Mannervik, 1985). Catalase activity in erythrocytes was assayed by a method described by Goth, 1991. The rate of

dismutation of H_2O_2 to water and molecular oxygen is proportional to the activity of catalase. Therefore, the sample containing catalase is incubated in the presence of a known concentration of H_2O_2 . After incubation for exactly one minute, the reaction is stopped with ammonium molybdate. The amount of H_2O_2 remaining in the reaction is then determined by the oxidative coupling reaction between molybdate and H_2O_2 (Goth, 1991). All reagents used were of analytical reagent grade. DTNB and thiobarbituric acid were obtained from sigma chemicals, St. Louis, MO., USA. Statistical analysis between controls and neonatal jaundice was performed by student t - test using the stat-view package. The data were expressed as mean \pm SD. $P < 0.05$ was considered as significant. Since our goals were to evaluate the differences of antioxidant status between neonatal jaundice and the healthy controls.

Results and Discussion

The number of neonates in healthy control group which are not jaundiced were 43 (22.87%) of the total neonates included and their TSB levels were found to be 0.65 ± 0.29 mg/dl., whereas, the remaining neonates which include 145 (77.13%) of the total cases were associated with the appearance of severe neonatal jaundice and their TSB levels were significantly elevated to 20.41 ± 5.13 mg/dl as compared with the control group ($p < 0.05$). There were few studies on the incidence of severe G6PD deficiency and the status of oxidative stress parameters in neonatal jaundice in Iraq. A total of 188 neonates were screened for erythrocyte G6PD enzyme activity. These samples were randomly tested for G6PD deficiency to determine whether or not this deficiency could play an important role in the development of neonatal jaundice. Of these subjects, 43 neonates (22.87%) showed a normal enzyme activity ($9.95 \pm$

1.75 U/g Hb) with normal TSB levels. Among the neonatal jaundice, only 14 of 145 cases (9.66%) with TSB level 24.38 ± 6.53 mg/dl was diagnosed and found to have severe G6PD deficiency which is the percentage of incidence in Diwaniyah Province : Iraq. Their mean \pm SD of G6PD activity levels were significantly decreased ($P < 0.05$) to 0.41 ± 0.3 U/g Hb as compared with the control G6PD activity identified 9.95 ± 1.75 U/g Hb (table-1-). Serum conjugated bilirubin (SCB) was also determined, and its mean \pm SD values in neonatal jaundice with severe G6PD deficiency were 0.094 ± 0.055 mg/dl which was significantly lower than that found in control group 0.21 ± 0.09 mg/dl ($P < 0.05$). These results confirm with other studies performed in Italy, and Taiwan which suggest that the G6PD-deficient neonates are at increased risk for jaundice. (Weng, et. al., 2002). Therefore, data presented in this study may probably suggest that severe neonatal jaundice may continuously cause of a problem in this region of Iraq, which show that those neonates with severe G6PD-deficiency who developed higher maximal TSB values had significantly lower SCB fractions. Conversely, those with lower SCB values at the time of sampling were at higher risk for the subsequent development of jaundice. Serum bilirubin profile demonstrated in the subsequently jaundice with severe G6PD-deficient neonates (high TSB, with low SCB) is a reminiscent of that seen in conditions of partial deficiency of the bilirubin conjugating enzyme UDP-glucuronosyl transferase1 A1 (UGT1A1), such as Gilbert's Syndrome. The primary site of the pathogenesis of jaundice therefore appears to be localized to a deficiency in bilirubin conjugation (Muraca, et. al. , 1987). As a result, G6PD-deficient neonates who become jaundice have bilirubin conjugation ability which is even more inefficient than that of the physiological immaturity of conjugation normally found in neonates. Those with an excessively immature bilirubin eliminating

capacity are more likely to develop jaundice than those with a more mature ability. This mechanism may exist to a certain extent in all neonates but may be exacerbated in the G6PD deficiency state because of increased hemolysis and the resultant additional bilirubin load (Kaplan, et. al., 1996). The results obtained also show that deficient bilirubin conjugation which was reflected by low SCB values measured, is a cardinal factor in the pathogenesis of G6PD deficiency associated with neonatal jaundice. In G6PD-deficient neonates who conjugate bilirubin less efficiently, jaundice is more likely to result. It is unknown at present time whether the previous observations related to hemolysis and bilirubin production (Kaplan, et. al., 1996), or the

deficient serum conjugated bilirubin fractions described above are unique to Sephardic Jews with G6PD Med or whether they have global implications for the hundreds of millions of people worldwide estimated to have G6PD deficiency (Beutler, 1994). Additional study of the pathophysiology of this process may lead to improved therapeutic or prophylactic interventions in the clinical management of G6PD deficiency associated neonatal jaundice. The results obtained indicated that there is a significant negative correlation between the decreased in G6PD activity levels and TSB concentrations elevated in neonatal jaundice with severe G6PD deficiency ($r = -0.367$, $P < 0.05$) but not in control individuals (Table-1-).

Table 1: G6PD activity levels, TSB, SCB concentrations and oxidant-antioxidant profiles in normal and full-term male neonatal jaundice with severe G6PD deficiency in Diwaniyah Province: Iraq.

Parameters	Control n = 43	Neonatal Jaundice with severe G6PD deficiency , n = 14	P value
G6PD , U/g Hb*	9.95 ± 1.75	0.41 ± 0.3	< 0.05
TSB, mg/dl	0.65 ± 0.29	24.38 ± 6.53	< 0.05
SCB, mg/dl	0.21 ± 0.09	0.094 ± 0.055	< 0.05
GSH, μM/g Hb*	5.66 ± 1.09	2.34 ± 0.94	< 0.05
MDA, nM/g Hb	37.4 ± 5.77	74.79 ± 11.95	< 0.05
G-Red, U/g Hb*	10.67 ± 1.48	6.29 ± 1.46	< 0.05
G-Px, U/g Hb*	39.77 ± 5.38	49.03 ± 10.25	< 0.05
Catalase, kU/g Hb*	98.42 ± 6.82	72.61 ± 11.4	< 0.05

***Note:** All the enzyme activity units were defined previously.

The mechanism of the relationship between G6PD activity and neonatal jaundice is not clear. The presence of another genetic factor has been postulated in the pathogenesis of neonatal jaundice in G6PD deficiency. Kaplan, et. al., (1997) reported that UGT1A1 gene mutation, diminishing the activity of the conjugated enzyme UGT1A1, was associated with neonatal jaundice in severe G6PD deficiency. Weng, et. al., in (2002) reported that the expression of heme oxygenase-1, a rate-limiting enzyme in the production of bilirubin and inducible under the exposure to oxidative stress, was

increased in G6PD deficiency. Recent studies suggest that bilirubin was a strong endogenous antioxidant. Therefore, it is reasonable to suggest that the neonatal jaundice caused by increased heme oxygenase-1 in G6PD deficiency is the consequence of genetic interaction to compensate the decreased antioxidant activity. Therefore, the low levels of G6PD activity in male infants may play a role in the interaction of different genes, such as UGT1A1 and heme oxygenase-1, and subsequently aggregate the high TSB levels. Erythrocytes are firstly associated to increase the activity of free

radical oxidation and to exhaust their compensatory potential. Previous studies on erythrocyte antioxidant capacity and human disease relation showed that some changes in activities of the antioxidant enzymes in the cell may occur (Karatat, et. al., 2003). In this study, the mean \pm SD of erythrocyte GSH, MDA concentrations and G-Red, G-Px and catalase activity levels were determined in Iraqi neonatal jaundice with severe G6PD deficiency and compared with the control group. There was a significant decrease in the erythrocyte GSH levels in neonatal jaundice with severe G6PD deficiency ($P < 0.05$) in Diwaniyah Province as compared with the control group (Tables-1-). Whereas the erythrocyte lipid peroxidation product MDA levels was significantly increased ($P < 0.05$) and reached to 74.79 ± 11.95 nM/g Hb as compared with control values 37.37 ± 5.77 nM/g Hb. The activities of erythrocyte antioxidant enzymes G-Red, and catalase were significantly decreased in neonatal jaundice with severe G6PD deficiency ($P < 0.05$), whereas the activity of the other antioxidant enzyme G-Px is significantly increased ($P < 0.05$) as compared with control group (Table -1-). The data obtained from this study indicate that there is increases in free radical generation and the antioxidant defense mechanism is impaired which is in agreement with other report (Ostrea, et. al., 1985) that concerned with neonates jaundice; and other studies published in Italy (Casado, et. al., 1995; Tanphaichitr, et. al., 1995), while it is in disagreement with others seen in Kurdish Jews, China and Saudi Arabia (Sodeinde, 1992; Du, 1992; Al-Omran, et. al., 1999). The lipid peroxidation end product malondialdehyde (MDA) levels have been increased significantly in erythrocytes of neonatal jaundice with severe G6PD deficiency than that found in control group. This may indicate the presence of increased oxidative stress. Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the

excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including cell membrane lipids. The raised MDA level in severe G6PD-deficient neonates reflects the oxidative injury due to neonatal jaundice, which is attributed to free radical generation that abstracts of hydrogen atoms from lipoproteins causing lipid peroxidation, of which MDA is the main product (Halliwell, 1994). The membrane phospholipids, specifically polyunsaturated fatty acids are converted to MDA, which can be analyzed by reactivity to thiobarbituric acid (Ostrea, et. al., 1985). It was also observed that there is a significant decrease in the levels of erythrocyte reduced glutathione (GSH), in neonatal jaundice with severe G6PD deficiency when compared to controls (Table-1-). GSH is important in chain breaking antioxidants responsible for scavenging the free radicals and suppression of peroxidation in aqueous and lipid region of the cell (Halliwell, 1994). The decrease in the levels of GSH observed may be due to the increased turnover, for preventing oxidative damage in these neonates suggesting an increased defense against oxidant damage in neonatal jaundice. Similar reports that were associated with a decreased levels of GSH concentrations in neonatal jaundice were reported by various studies (Turgut, et. al., 2004). In this study, the erythrocyte antioxidant enzyme glutathione peroxidase was slightly elevated in neonatal jaundice with severe G6PD deficiency as compared with that found in control group (Tables-1-). G-Px is an oxidative stress inducible enzyme that plays a significant role in the peroxy scavenging mechanism and in maintaining functional integration of cell membranes (Sullivan and Newton, 1988). The rise in its activity levels could be due to induction to counter the effect of increased oxidative stress. G-Px provides an effective protective mechanism against cytosolic

injury because it eliminates H_2O_2 and lipid peroxide products by reduction reactions utilizing GSH. Decrease in the activities of antioxidant enzyme status was reported in various studies (Majumder, et. al., 1995; Kilic, et. al., 2004). In the present study, it was also observed a significant decrease in the activity levels of catalase and G-Red in

neonatal jaundice with G6PD deficiency as compared to controls (Tables -1-). Catalase is the enzyme which protects the cells from the accumulation of H_2O_2 by dismutating it to form water and oxygen or by using it as an anti-oxidant in which it works as a peroxidase.

Table-2- The following table indicated the correlation between G6PD activity levels with each of TSB levels and the different antioxidant biomarkers and lipid peroxidation end product (MDA) in neonatal jaundice with severe G6PD deficiency in Diwaniyah province : Iraq.

TSB and oxidative stress parameters	Full-term Neonatal jaundice TSB \geq 15 mg/dl			
	Mean \pm SD	G6PD activity Mean \pm SD U/g Hb	P Value	r Value
TSB conc., mg/dl	24.38 \pm 6.53	0.41 \pm 0.3	< 0.05	- 0.367
GSH conc. , μ M/g Hb	2.34 \pm 0.94	0.41 \pm 0.3	< 0.005	+ 0.609
MDA conc., nM/g Hb	74.79 \pm 11.95	0.41 \pm 0.3	< 0.005	- 0.600
G-Red activity , U/g Hb	6.29 \pm 1.46	0.41 \pm 0.3	< 0.05	+ 0.819
G-Px activity, U/g Hb	49.03 \pm 10.25	0.41 \pm 0.3	< 0.05	- 0.562
Catalase activity , kU/g Hb	70.69 \pm 17.04	0.41 \pm 0.3	< 0.05	+ 0.541

Reports in the literature have shown that the decreased activity of G-Red is the result of changes in normal activity of G6PD whose deficiency may limit NADPH synthesis (Griffith, 1999). The relationship between G6PD deficiency, oxidant damage and mechanical impairment is quite expected and well known one. In RBC, like in most other cells, the only source of NADPH is HMP-shunt (Beutler, 1994). Glucose-6-phosphate (G6P) is converted into 6-phospho-gluconolactone, catalyzed by G6PD, and accompanied by a reduction of $NADP^+$ into NADPH. A sufficient amount of NADPH is essential for the integrity of RBC, because it reduces glutathione, which plays important roles in the anti-oxidant defense mechanisms of RBC (Chan, et. al., 1999). NADPH is the coenzyme of G-Red enzyme which regenerate GSH from GSSG, which in turn takes part in the conversion of H_2O_2 (Beutler, 1994). Therefore, the deficiency of G6PD leads to increased oxidant Reactions of bilirubin involving free

radicals or toxic oxygen reduction products have been well documented: unconjugated bilirubin scavenges singlet oxygen with high efficiency, reacts with superoxide anions and peroxy radicals, and serves as a reducing substrate for peroxidases in the presence of H_2O_2 or organic hydroperoxides (Stocker and Ames, 1987). The results showed that positively significant correlation was found between G6PD activity levels with GSH concentrations ($r = + 0.609$, $p < 0.005$), whereas a negative significant correlations was found between G6PD activity levels and MDA concentrations ($r = - 0.600$, $p < 0.005$). The results of each G6PD and G-Red activity levels identified in neonatal jaundice with severe G6PD deficiency indicated that the decreased levels of G6PD activity was significantly positively correlated with the decreased levels of G-Red activity ($r = + 0.819$, $p < 0.05$), and the results obtained from G6PD and G-Px activity determination indicate that G6PD activity levels were negatively significant correlation with the elevated

levels of G-Px activity ($r = -0.562$, $p < 0.05$). The data obtained from this study also indicated that decreased G6PD activity levels was positively significant correlation with the reduced catalase activity levels ($r = + 0.541$, $p < 0.05$) as indicated in (Table-2). The antioxidants are classified into: primary, secondary and tertiary defense. The primary antioxidants mechanism work by preventing the formation of new free radical species which include SOD, G-Px and metal-binding proteins (e.g. ferritin or ceruloplasmin). Secondary antioxidants trap radicals thereby preventing chain reactions. These include vitamin E, vitamin C, beta-carotene, uric acid, bilirubin and albumin. Tertiary antioxidant repair biomolecules damaged by free radicals. These include DNA repair enzymes (Jacob, 1995). In the present study, various enzymatic and non-enzymatic antioxidant defense system have been determined in neonatal jaundice with severe G6PD deficiency and compared with that identified in control full-term neonates. G-Red, and catalase activity levels, which are well known antioxidants enzymes were significantly lower in neonatal jaundice with severe G6PD deficiency as compared with that found in control group, whereas GSH concentration levels was also decreased. Interestingly, the other activity levels of antioxidant enzyme G-Px and the lipid peroxidation end product MDA, were increased in neonatal jaundice with severe G6PD deficiency. There was also a significant positive correlation between MDA and TSB in severe G6PD-deficient neonates. This study, revealed the presence of an association between serum oxidant / antioxidant parameters in full-term male neonatal jaundice with severe G6PD deficiency in Diwanayah Province of Iraq. In a healthy human being, the formation and inactivation of reactive oxygen species are balanced at a level at which the compounds can play their physiological role without any toxic effects. This balance

can be unstable in the neonatal period following rapid changes in tissue oxygen concentration, immature antioxidant mechanism and considerable neonatal developmental changes in antioxidants. This deterioration is especially evident in the presence of oxidative stress such as phototherapy. Neonatal jaundice affects 60% of full term infants and 80% of preterm infants in the first 3 days after birth (Melton and Akinbi, 1999). Although transient, the condition accounts for up to 75% of hospital re-admissions in the first week after birth (Briton, et. al., 1994). Antioxidant activity in the serum of term neonates is lower than that of adults and is still lower in preterm and low birth weight babies as compared to term babies (Sullivan and Newton, 1988). Red blood cells are extremely susceptible to lipid peroxidation since they are rich in unsaturated membrane lipids, have rich supply of oxygen and transitional metal catalysts. Neonatal erythrocyte membrane is more susceptible to oxidative damage due to its predominant pro-oxidant potential (Jain, 1989). The erythrocytes are particularly prone to the free radical damage since the membrane lipids are very rich in polyunsaturated fatty acids which play an essential role in generating free radicals. Free radicals, primarily the reactive oxygen species, superoxide and hydroxyl radicals which are highly reactive having an unpaired electron in an atomic or molecular orbit are generated under physiological conditions during aerobic metabolism. As free radicals are potentially toxic, they are usually inactivated or scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids. Alteration in the oxidant – antioxidant profile is known to occur in neonatal jaundice (Turgut, et.al, 2004). Moreover the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation.

Conclusions

Glucose-6-phosphate dehydrogenase deficiency is a major public health problem. Geographically, it is heterogenous among Iraqi population. High percentage of incidence of severe G6PD deficiency with TSB \geq 15 mg/dl was observed Diwaniyah Province : Iraq (9.66%). The results obtained concluded that severe neonatal jaundice continues to be a problem in Diwaniyah Provinc. The data obtained indicate that severe G6PD deficiency play an important role as a common etiologic factor in neonatal jaundice in this region of Iraq. Decreased levels of GSH, catalase and G-Red were observed in neonatal jaundice with severe G6PD deficiency. Increased levels of G-Px and lipid peroxidation end product MDA were observed which indicate an increased in ROS generation due to different causes. Negative correlations were observed between G6PD activity and each of MDA and G-Px in neonatal jaundice with severe G6PD deficiency whereas, positive correlations were observed between G6PD activity and each of GSH, G-Red, and catalase .

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