Role of Oxidant/Antioxidant Status in the Pathogenesis of Iron-Deficiency Anemia: Case Study on Children of Qalyubiyya and Minoufiya Governorates, Egypt

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ABSTRACT:

Iron- deficiency anemia (IDA) is anemia due to insufficient iron. It is one of the major causes of morbidity and mortality worldwide affecting people of all ages in both developed and developing countries. The present study aims to investigate the role of oxidative/antioxidant status in the pathogenesis of IDA through a case study on children of Qalyubiyya and Minoufiya Governorates, Egypt. 119 with IDA and 150 healthy control, boys and girls, infants and young children, aged zero months to 6 years, were selected for the study from Rural Health Units and Maternity and Child Care Centers, Qalyubiyya and Minoufiya Governorates, Egypt. Based on hematological and biochemical analysis, the mean hemoglobin (Hb) level, mean corpuscular volume (MCV), reticulocyte hemoglobin content, serum iron and serum ferritin were significantly lower in infants and young children with IDA than healthy control. The opposite direction was observed for the red blood cell distribution width (RDW). The antioxidant defense system parameters including [enzymes (glutathione peroxidase, GSH-Px; glutathione reductase, GSH-Rd; superoxide dismutase, SOD and catalase, CAT) activities and none-enzymatic levels (glutathione, GSH and vitamins A, C and E)] of infants and young children with IDA were significantly ($P \leq 0.05$) lower. While the oxidative stress parameters thiobarbituric acid reactive substances, TBARS and nitric oxide, NO_2 were significantly higher than the control group. In conclusion, oxidant / antioxidant status may play a role in the Pathogenesis of Iron-Deficiency Anemia.

Keywords: *Hematologic markers- antioxidant (enzymes; vitamins) - oxidative stress*

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INTRODUCTION

Martins *et al.,* (2001) defined iron deficiency anemia (IDA) initially as a decrease for hemoglobin (Hb) or red blood cells (RBCs) in the blood. According to the basis of the 1999 –2002 US National Health and Nutrition Examination Survey, IDA is generally characterized by a Hb level of less than 11 g/dl plus a measure of poor iron status among children aged 12-35 months **(Martins *et al.,* 2001; CDCP, 2008 and Cusick *et al.,* 2008).** Reduction in serum iron concentration causes insuffi-cient Hb synthesis with subordinate decreasing in erythrocyte proliferation. In addition, reduction in red cell survival may be present in patients with IDA **(Hafez *et al.,* 1986 and Cusick *et al.,* 2008).** IDA is considered one of the major health problems. According to the World Health **Organi-zation, (2008)**, ane-mia affects 1.62 billion people, which corresponds to 24.8% of the world population. The highest prevalence of anemia is in preschool - age children (47.4%), and the lowest prevalence is in men (12.7%) as well as the population group with the greatest number of individuals affected is non-pregnant women (468.4 million). In Egypt, anemia was a public health problem where the prevalence of it reached 40% **(EDHS, 2005)**. The study by **Elashiry *et al.,* (2014)** reported that anemia by third trimester of pregnancy represents major health problem, the risk factors include personal, dietary, and some aspects related to outcome healthcare delivered at this stage. In addition, **Tawfik *et al.,* (2015)** reported that iron IDA (low Hb and low ferritin) was recognized among 18.5% through 4526 households from eleven Egyptian governorates, with high prevalence for mothers (25.1%). Furthermore, **Al Ghwass *et al.,* (2015)** found that the high frequency of IDA is a severe public health problem in the Egyptian child, especially in rural areas, those from low social class and

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those of low maternal educational level.

Oxidative stress (OS) is defined as a shift in balance between oxidant/antioxidant in cells and tissues in favor of oxidants (**Toshikazu and Yuji, 2002**). It is used to describe a number of chemical reactions involved in the production of free radicals known as reactive oxygen species (ROS), such as singlet oxygen (O^-), superoxide radicals ($\cdot O2-$),, hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$) that are potentially able to induce cellular injury (**Halliwell, 1994 and Kim *et al.,* 2006).** When ROS products increased they start showing harmful effect on biological molecules such as lipids, proteins, nucleotides / DNA, etc. (**Aust and Eveleigh, 1999; Evans et al., 2005; Somogyi *et al.,* 2007 and Rahman *et al.,* 2012**). Through oxidation reaction free radicals cause damage to these molecules, disturbing their normal functions, and may therefore contribute to a variety of diseases (**Somogyi *et al.,* 2007).** Such diseases include diabetes mellitus, heart diseases, obesity, respiratory distress syndrome, and ischemic diseases, neuro-degenerative diseases such as Parkinson's disease and Alzheimer's disease, and cancer (**Chaitanya *et al.,* 2010 and Elmaadawy *et al.,* 2016).** Major defense mechanisms versus ROS include enzymatic (glutathione peroxidase, GSH-Px; glutathione reductase, GSH-Rd; superoxide dismu-tase, SOD; and catalase, CAT as well as non-enzymatic systems (reduced glutathione, GSH; vitamins A, C and E) (**Halliwell, 1991 and 1994).** In addition to these major enzymes and non-enzymes, other antioxidants, including heme oxygenase-1, and redox proteins have also been found to play crucial roles in the body antioxidant defenses (**Esra *et al.,* 2012 and Rahman *et al.,* 2012**).

In IDA subjects, increased OS was reported in several studies, in which the oxidative status was evaluated

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using measurement of oxidants and antioxidants (**Kumerova et al., 1998; Kurtoglu et al., 2003 and Mehmet et al., 2011**). Such studies reported that oxidants were increased and antioxidants were decreased, and as a result, the oxidant / antioxidant balance shifted toward the oxidative side in patients with IDA. In addition, OS is known to be a positive contributor for anemia, giving its effects on lipid peroxidation, antioxidant vitamins and DNA damage (**Saadet et al., 2013 and Nirjala and Kora, 2014**). In spite of the above, searches indicated, that literature offer limited data on oxidative stress and antioxidant defense in patients with IDA. Therefore, the present study aimed to investigate the potential roles of oxidant/antioxidant status in the pathogenesis of IDA through a case study on children of Qalyubiyya and Minoufiya Governorates, Egypt.

MATERIALS & METHODS
Chemicals and Equipment's

GSH and vitamins (A, C and E) standard were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals and buffers, except stipulated, were purchased in analytical grade from Al-Gomhoria Company for Trading Drugs, Chemicals and Medical Requirements, Cairo, Egypt.

Liquid chromatograph (LC) system (Thermo Separation products, San Jose, CA) was used for GSH fractions and vitamins determination. The system consists of a Consta Metvic 4100 pump, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer. Detector, Spectra System FL 3000, a PC 1000 system software and columns (Alltech, Deerfield, IL) Spherosorb ODC-2 (5 µm, 150 x 4.6 mm I.d.). For GSH fractions; a reversed-phase water Adsorbosil C_{18} (5 µM, 100 mm × 4.6 mm I.d.) for vitamin C; and normal Ultrasphere Si (5 µM, 250 mm × 4.6 mm I.d.) for vitamins A and E. Also, absorbance (Labo-

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med. Inc., spectrophotometer, CA) and fluorescence (Schematzu fluorescence apparatus, Japan) were used for different assays.

Study protocol

In the present study, a protocol was applied according to the guidelines laid down in the declaration of Helsinki and all procedures involving infants / children families' were approved in advance by the Ethical Committee of Scientific Research, Faculty of Home Economics, Minoufiya University, Shebin El-Kom, Egypt. Oral informed consent was received from infants /children families, witnessed and formally recorded. Survey study was conducted from January 2020 to August 2020, in the Rural Health Units and Maternity and Child Care Centers, Qalyubiyya and Minoufiya Governorates, Egypt. These centers serve most children from low and middle socioeconomic standards. 241 children aged 0 to 6 years who were searching medical advice were enrolled in the study. Online statistical calculation was used for sample size determination guided by power test of 80%, confidence level of 95%, and α error of 5%.The sample size was calculated to be 279 young children. The exclusion criteria included iron treatment before or during the study period, any history of chronic illness and recent blood transfusion. A field pretested interviewing questionnaire was used for data collection from the infants/children families which covering the following topics: child sex, age, family size and residence. Socio-demographic status of children and their families, socio-economic score, which contained social variables including fathers' education and work (score 2-10), mothers' education and work (scores 1-10) and crowding index (scores 1-5). The total score were calculated according to mentioned by **Fahmy and El-Sherbini, (1983)** as follow: score from 19-25 means high social class, score from 12 to 18 means

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middle social class and score below 12 means low social class.

Hematological analysis

Five ml of blood from normal and anemic individuals was collected in a sterile vial containing ethylene diaminetetraacetic acetate (EDTA) as anticoagulant. Fresh whole blood was used for meaning of Hb and the rest of the blood was centrifuged at 1500 Xg for 10 minutes, plasma was withdrawn and used for analysis of blood biochemical parameters and antioxidant vitamins. The erythrocyte (RBC's) residue was washed with three successive portions of sodium chloride solution (0.9%) and then hemolyzed with deionized water for 30 minutes. Hemolysate was then centrifuged at 105,000 g for 30 minutes, and the supernatant fractions was transferred to a clean test tube and analyzed for antioxidant enzymes **(Stroev and Makarova, 1989).**

The Hb level in whole blood samples was determined by using cyanmethemoglob in

method according to **Villanova, (1994)**. Anemia was diagnosed when Hb concentrations below the values adjusted for age groups **(Wonke *et al.,* 2007)**. In infants from 6 months to 6 years mild, moderate and severe anemia was diagnosed if Hb level was 10-11, 7-9.9, or below 7 g/dl, respectively **(Bermejo and Garcia-Lopez, 2009).** Microcytosis was diagnosed when the MCV was below 80 fl and MCH below 27 pg **(Bermejo and García-López, 2009).** Serum iron (Fe) content samples were determined by the adaptation the method mentioned by **Singh *et al.,* (1991**) using of atomic absorption spectrophotometer, type Perkin - Elmer, Model 2380, Tokyo, Japan. The plasma ferritin concentrations and hematocrit value were assayed using specific Kits (Al-Gomhoria Company for Drugs, Chemicals and Medical Instruments, Cairo, Egypt) according to the methods mentioned in **Tietz, (1999).** The complete blood count was

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done using Coulter 1660 to determine the erythrocyte indices [mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and red cell diameter width (RDW)].

Antioxidant enzymes

Glutathione peroxidase (GSH-Px) and catalase (CAT) activities in hemolyzed RBC lysates were assayed by the methods of Splittgerber and **Tappel, (1979), and Aebi, (1974),** respectively. Super-oxide dismutase (SOD) activity in hemolyzed RBC lysates was determined by Ransod kit (Randox laboratories Limited, Germany). GSH-Rd activity in hemolyzed RBC lysates was determined by the method recommended by the International Committee for Standardization in Hematology **(ICSH, 1979).**

Antioxidant vitamins

All vitamins A, C, and E were extracted and analyzed by HPLC techniques as described by the methods of **Epler** *et al.,* **(1993); Moeslinger** *et al.,* **(1994) and Hung,** *et al.* **(1980),** respectively. Under such chromatographic separation conditions, the Mean ±SD values of vitamins A, C and E recoveries were 87.78±3.56, 82.17±6.24, and 93.04±2.11, respectively.

Nitrite and Nitrite/nitrate determination

Nitrite and nitrite/nitrate determination were determined flourometric such as described by **Misko** *et al.,* **(1993).**

Thiobarbituric acid reactive substances (TBARS) content

The lipid peroxidation product, TBARS /malon-dialdehyde (MDA) in plasma, was determined according to **Buege and Aust, (1978).**

Statistical analysis

Data were summarized using mean± SD and range for quantitative variables and number and percentage for qualitative variables. For statistical analysis, the packaged program, Statistical package for social sciences

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(SPSS) for windows (version 13.0, Chicago, IL) and MINITAB-12 computer program (Minitab Inc., State College, PA) were used.

RESULTS &DISCUSSION

- *Socio-demographic characteristics of the studied group*

The socio-demographic characteristics of the studied group are shown in Table (1). From such data, it could be noticed that among 279 infants/children enrolled, 38 (13.62%) children were not analyzed because of unavailability of consent for study or inadequate sample. The remaining enrolled 241 children aged 0 months to 6 years with a mean age of 3.47 ± 2.01 years. They were 112 (46.47.30%) boys and 129 (53.53%) girls. According to the biochemical iron status, 91 children (37.76%) were anemic. The largest sample size was in the rural area (59.34%) while the rest (40.66) form the urban area. The highest percentage (45.64%) of the sample lies in middle social class while

related to the levels of mothers education, 35.27% of the sample lies in secondary school group.

Hematologic markers of iron deficiency anemia (IDA) and healthy controls groups

Hematologic markers of IDA and healthy controls groups are shown in Table (2). From such data it could be noticed that the mean hemoglobin (Hb), the concentration of oxygen carrying protein, level, mean corpuscular volume (MCV) and Reticulocyte hemoglobin content (RHC) of normal children were significantly decreased in compare IDA children. That is meaning the Hb, MCV and RHC were decreased in IDA by rate of 50.53, 24.06 and 15.00%, respectively. The opposite behavior was observed for the red blood cell distribution width (RDW) was increased significantly (p<0.01) of normal group compared to IDA group. Such data are in accordance with that observed by **Ann** *et al.,* **(2002)** who

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reported that measurement of Hb is a more sensitive and direct test for anemia than others tests such measurement of hematocrit (Hct) and the percentage of whole blood that is occupied by RBCs. In general, anemia is defined as Hb values less than 11.0 g/dl for children 6 months to 2 years of age. Hb measurement is inexpensive, readily available test for anemia and is used most commonly to screen for iron deficiency (ID). However, Hb is a late marker of ID, is not specific for IDA, and is less predictive as the prevalence of IDA decreases. MCV, the average volume of RBCs, is reported in automated analyses, but it also can be calculated as the ratio of Hct to RBC count. MCV is useful for categorizing anemia as microcytic, normocytic, and macrocytic. Additionally, RDW measures variations in the size of RBCs and increases with ID. On the other side, the reticulocyte count measures circulating immature RBCs and decreases with ID. However, the RHC increases with blood loss. In severe cases of ID anemia coupled with blood loss, the RHC may be slightly elevated. This parameter often is used for assessing the response to iron supplements (**Wu *et al.,* 2002**). Also, **Booth and Aukett, (1997) and Ann *et al.,* (2002)** reported that RDW is not as useful alone as a screening test, but it is used frequently in conjunction with MCV to differentiate among various causes of anemia because of its relatively low specificity. For example, RDW is high in IDA, but low in thalassemia minor.

Biochemical markers of IDA and healthy control groups

Biochemical markers of IDA and healthy control groups are shown in Table (3). From such data it could be noticed that the mean serum iron, serum ferritin and transferrin saturation of normal children were sig-nificantly (p≤0.001) decreased versus of IDA group. That is meaning the serum iron, serum ferritin and transferrin

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saturation were decreased in IDA by rates of 76.28, 93.86 and 79.22%, respectively. The opposite behavior was observed for the Zinc protoporphyrin /heme, which recorded 35.99 µmol/mol in normal infants and significantly (p≤0.01), increased to 72.89 µmol/mol (rate, 102.53%) in IDA group. In similar study, **Fairbanks, (1991)** mentioned that serum iron concentration can be measured directly and generally decreases as iron stores are depleted. Also, **Oski, (1993)** found that serum iron may not reflect iron stores accurately because it is influenced by several additional factors, including infection, inflammation, diurnal variation and iron absorption from meals. On the other side, ferritin is a storage compound for iron and serum ferritin levels normally correlate with total iron stores. In this manner, **Ann *et al.,* (2002)** found that iron stores are depleted; serum ferritin levels decline and are the earliest marker of ID. So,

serum ferritin has high specificity for ID, specifically when combined with other markers such as Hb. Also, **Fairbanks, (1991) and Oski, (1993)** reported that test is expensive and has limited availability in a clinic setting; therefore, it is not used commonly for screening ID. Additionally, serum ferritin can become elevated as a common factor in many diseases such as inflammation, chronic infection and other.

Zinc protoporphyrin / heme (ZPP/H) ratio is formed generally when zinc is incorporated into protoporphyrin instead of iron during the final step of heme synthesis. In normal conditions, the reaction of protoporphyrin with iron predominates, but when iron is in short conditions, the production of ZPP/H increases subsequently the ZPP/H ratio becomes increased (**Wu *et al.,* 2002).** The study of **Braun *et al.,* (1996)** reported that ZPP/H ratio reverberate s iron status during hemoglobin biosynthesis and detects ID

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before the incipience of anemia. Although ZPP/H can be measured by using clinic-based methods ZPP is elevated with other cases than anemia such lead poisoning and chronic disease. Such investigation making ZPP/H less useful for the diagnosis of anemia (**Wu** *et al.,* **2002 and Eckerstrom** *et al.,* **2020).** In addition, **Rettmer** *et al.,* **(1999)** reported that increase in the ZPP/H ratio is demonstrated to be a sensitive, specific, and cost-effective test for identifying pre-AID in a community pediatric practice.

On the other side, transferrin saturation (Tf sat) indicates the proportion of occupied iron-binding sites and reverberates iron transport rather than storage. In study of **Adams, (2015)** reported that low Tf sat implies low serum iron levels relative to the number of available iron-binding sites subsequently low iron stores. In addition, **Barton** *et al.,* **(2015)** reported that Tf sat decreases before anemia develops, but not nearly enough to identify iron depletion.

Changes in biological antioxidants of IDA and healthy control groups Glutathione fractions

RBC's glutathione fractions of IDA and healthy control groups are shown in Table (4). From such data it could be noticed that the mean of RBC's GSH and GSSG levels of normal children were 9.06 and 0.73 µmol/L which decreased to 5.01 (significantly, $p<0.01$) and 0.58 µmol/L ($p\leq0.05$) in IDA group, respectively. In addition, a fall in GSH fractions observed generally accompanied by a concomitant decreased in the ratio of GSH/GSSG. The mean GSH/GSSG value of normal children was 12.41, which significantly ($p\leq0.01$) dec-reased to 8.63 in IDA group, respectively. That is meaning the RBC's level of GSH, GSSG and GSH/GSSG were decreased in IDA group by the rates of 44.70, 20.55 and 30.46%, respectively.

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GSH has received high attention in terms of its biosynthesis, regulation, and various intracellular functions **(Reed and Beatty, 1980; Larsson *et al*., 1983 and Elmaadawy *et al.,* 2016).** Such functions includes as a key conjugate of electrophilic intermediates and as an important antioxidant in detoxification process. The antioxidant functions of GSH includes its role in the activities of GSH antioxidant enzymes (GSH-Px and GSH-Rd). In addition, GSH can apparently serve as a nonenzymatic scavenger of oxyradicals **(Almutairiy, 2020).** The study of **Di Giulio, (1991)** found that high effect of oxyradical - generating compounds effect on the redox status (GSH/GSSG) of cells and tissues. Similar findings are recorded for diseases other than IDA. **Lo et al., (1971),** reported first observation for hemolytic anemia associated with decreased concentration of GSH in Red Cells. They found that three cases of hemolytic anemia with

decreased concentration of GSH in red cells. Also, Nour **Eldine, (2013)** reported that significant reducing (p>0.05) in GSH fractions level in full term neonates diagnosed to have idiopathic hyper-bilirubinemia as compared with the control group.

Antioxidant enzymatic components:

Antioxidant enzymes activity in RBC's of IDA and healthy controls groups are shown in Table (5). From such data it could be noticed that significant reducing in GSH-Px, GSH-Rd, SOD and CAT activities in children diagnosed to have IDA as compared with the control group. The mean GSH-Px, GSH-Rd, SOD and CAT activities of normal group were significantly decreased versus IDA group, respectively. That is meaning the serum levels of GSH-Px, GSH-Rd, SOD and CAT activities were decreased in IDA patients by the rates of 22.04, 16.59, 32.21 and 26.34%, respectively.

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In general, SOD, CAT and the enzymes of the glutathione redox cycle i.e. GSH-Px and GSH-Rd are the primary intracellular antioxidants. They are considered preventive or primary, antioxidant as they prevent free radical chain reaction by decreasing the available concentration of free radical to initiate the process (**Heffner and Repine, 1989; Di Giulio, 1991 and Elmadaawy *et al.,* 2016).** Data of the present study confirmed that IDA causes moderate depression on the antioxidant defense potential of erythrocytes and thus, cells cannot cope with oxidant stress incurred by IDA injury. So, decreased GSH-Px, GSH-Rd, SOD and CAT activities in IDA patients are generally accepted. In similar study, **Hodgson and Fridovic (1975)** reported that ROS, especially hydrogen peroxide, inactivate SOD. In addition, GSH-Px is a selenium-dependent enzyme, and selenium concentration was significantly lower in-group with IDA (**Perona *et al.,* 1977**

and Yetgin *et al.,* 1992). Furthermore, **Jong-Ha *et al.,* (2009)** found decreasing in CAT activity in-group with IDA, which is because this enzyme is a protein containing iron.

Antioxidant vitamins

Antioxidant vitamins levels in plasma of IDA and healthy controls groups are shown in Table (6). From such data, it could be noticed that significant reducing in vitamins A, C and E levels in children diagnosed to have IDA as compared with the control group. The mean vitamins A, C and E levels of normal infants were 1.45, 50.54 and 31.78 µmol/L, which significantly decreased to 0.85, 38.0 and 23.45 µmol/L of IDA patients, respectively. That is meaning the serum levels of vitamins A; C and E levels were decreased in IDA group by the rates of 41.37, 24.81 and 26.21%, respectively. In similar study, **Nirjala and Kora, (2014)** found that higher OS before vitamin supplementation in IDA patients and after

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supplementation, lower lipid peroxidation and increased antioxidant vitamins were observed.

The reducing in antioxidant enzymes defense potential of erythrocytes was contrary with significant decreasing ($p \leq 0.05$) in antioxidant vitamins in children diagnosed to have IDA. According to these results, the decreasing in antioxidant vitamins in plasma could be attributed to their consumption in scavenge, quench and/or trap different ROS resulted from IDA injury. Vitamins include A, E and C, the non-enzymatic antioxidants that prevent or retards the oxidation of sensitive molecules found in the body. ID is regarded as the major cause of nutritional anemia, insufficient levels of vitamins A, B_1, B_{12}, C, E and folic acid has been found to be associated with this deficiency (**Kuypers et al., 1996**). In some clinical studies of **Chiu et al., (1989) and Gadjeva et al., (2005)** reported that vitamin E might have a role as a potential

erythropoietic agent in the IDA patients suffering from various types of hemolytic anemia, renal failure of hemodialysis, and borderline anemia. Also, vitamin E has the potential to be effectively used for preventing and/or treating some types of human anemia due to its supposed role in promoting erythropoiesis, improving the integrity and stability of RBC's membrane, and reducing the OS induced erythrocyte fragility and lysis resulting in increased RBC's survival and Hb level (**Isler et al., 2002; Nagababu et al., 2008 and Nirjala and Kora, 2014).**

Changes in blood levels of oxidants in IDA and healthy control groups

Blood levels of oxidants in IDA and healthy control groups are shown in Table (7). From such data, it could be noticed that significant increasing in blood oxidants, TBARS, NO_2 and NO_3/ NO_2 levels of children diagnosed to have IDA as compared with the control

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group. The mean of oxidants TBARS, NO_2 and NO_3/NO_2 levels of normal infants were 1.87, 2.01 and 3.26 nmol/l which significantly increased to 2.31, 2.61 and 3.97 nmol/l in IDA group, respectively. That is meaning the blood levels of oxidants TBARS, NO_2 and NO_3/NO_2 levels were increased in IDA patients by the rates of 23.52, 29.85 and 21.78%, respectively. In similar study, **Nirjala and Kora, (2014)** found that higher OS before vitamin supplementation in IDA patients and after supple-mentation, lower lipid peroxidation was observed. Also, **Nour Eldine, (2013)** reported significant ($p \leq 0.05$) increased in TBARS, NO_2 and NO_2/NO_3 levels in plasma of full term neonates diagnosed to have idiopathic hyper-bilirubinemia. In vitamin C administration group, a sig-nificant decreasing was recorded with TBARS, NO_2 and NO_2/NO_3. The present data with the others recorded accompanied by a concomitant reduce in enzymatic and nonenzymatic antioxidants, high concentrations of different oxidants include TBARS and nitric oxides (nitrite, NO_2 and nitrate, NO_3) as established in the present study in full term neonates diagnosed to have idiopathic hyperbilirubinemia (before and after treatment with vitamin C). In our opinion, if there were no change in the antioxidant defense system of children diagnosed to have IDA, it would be difficult to observe high concentrations of TBARS and nitric oxides. In this vein, **Yoshihito, (2012)** reported that OS can damage specific molecular targets (lipids, proteins, nucleotides, etc.), resulting in cell dysfunction and /or death. OS is known to contribute to the pathogenesis of several here-ditary disorders of erythro-cytes, including sickle cell anemia, thalassemia, and glucose-6-phosphate dehydro-genase (G6PD) deficiency.

The possible signify-cance of malonaldehyde (MDA), one of the most important compounds in

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TBARS and major products of the oxidation of poly-unsaturated fatty acids, on human health has been stimulated by reports that are mutagenic and carcinogenic compound **(Shamberger *et al*., 1974 and Mahran *et al.*, 2018).** On the other side, nitric oxide synthase catalyzes the conversion of L-arginine to citrulline and highly reactive free radical species, nitric oxide (NO) **(Manahan, 1989)**. Nitric oxide, in turn, can react with molecular oxygen and water to form nitrite (NO_2) and nitrate (NO_3) with hemoglobin (Hb) to form iron-nitrosyl adducts and/or nitrate in blood, with superoxide anion to make nitrate, and with the amino and thiol groups of protein to produce nitrosylated species **(Manahan, 1989 and Misko *et al*., 1993).** The excess production of nitric oxides (NO_2 and NO_3) has been implicated in the pathogenesis and tissue destruction of a growing number of immune-logical and inflammatory diseases including septic shock, arthritis, graft rejection,

obesity and diabetes **(Jacob *et al*., 1992; Aly, 2017 and Almutairiy, 2020).**

Correlation analyses of the groups

In the intra correlation analyses, important differences were found because of IDA. Data in Table (8) indicated highly significant positive correlations between the serum iron ($r^2 = 0.8523$ at $P \leq 0.001$), serum ferritin, ($r^2 = 0.8874$ at $P \leq 0.001$) GSH ($r^2 = 0.8150$ at $P \leq 0.01$) and Hb of the groups. In addition, most important highly significant negative correlations established were between the oxidants (TBARS, $r^2 = -0.6865$ at $P \leq 0.01$ and nitric oxides, -0.7517 at $P \leq 0.05$) and Hb. These might be importance because they indicated that the factor(s) leading to increase antioxidants (GSH) in the cells might also cause increasing in the levels of Hb. Opposite direction was recorded for other factor(s) leading to oxidant stress and peroxidation in the cells might cause lowering in the levels of

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plasma Hb. Therefore, blood oxidants such TBARS and NO_2 are confirmed to contribute to the pathogenesis of several disorders of erythrocytes including IDA.

CONCLUSION

The high frequency of IDA is a severe public health problem in developing countries including Egypt. In addition, the IDA injury induced oxidative stress, which leads to the formation of ROS and/or other compounds. This is confirmed by the results of this study, which showed that IDA injury causes' reduction in the antioxidant defense system, enzymes (GSH-Px, GSH- Rd, SOD and CAT) and non-enzymes (GSH and Vitamins A, C and E) in serum as well as elevations in blood oxidants (TBARS and NO2). The results of our study support the role of oxidant/antioxidant status in the pathogenesis of IDA. Subsequently, antioxidant defense system including enzymatic and nonenzymatic and oxidants in blood should be taken in our consideration as important biochemical indicators during IDA assessment and/or diagnosis. In addition, health care providers to all infants from 6 months to 6 years should advise iron-rich foods in conjunction with anti-oxidant vitamins supple-mentation therapy.

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Table 1. Socio-demographic characteristics of the studied group

Variables	Studied group	
	Number (241)	Percentage (%)
Sex		
− Male	112	46.47
− Female	129	53.53
Health status		
− Normal	150	62.24
− IDA	91	37.76
Residence		
− Urban	98	40.66
− Rural	143	59.34
Age (years)		
− <1	32	13.28
− 1	26	10.79
− 2	30	12.45
− 3	29	12.03
− 4	52	21.58
− 5	31	12.86
− 6	41	17.01
Family size (person)		
− 1	31	12.86
− 2	61	25.31
− 3	41	17.01
− 4	44	18.26
− 5	39	16.18
− >5	25	10.37
Social class		
− Low	85	35.27
− Middle	110	45.64
− High	46	19.09
Level of mothers education		
− Illiterate/can read and write	54	22.41
− Primary/preparatory	61	25.31
− Secondary school	85	35.27
− University or higher	41	17.01

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Table 2. Hematologic markers of IDA and healthy controls groups

Parameters		Normal group (n=150)	IDA group (n=91)	Statistical analysis
Hemoglobin Hb (g/dl)	Range	12.08 -15.44	6.01-8.82	p≤0.001
	Mean ± SD	14.09±0.97	6.97±1.11	
	% of change	------	-50.53±3.67	
Red blood cell distribution width RDW (%)	Range	11.08-13.87	16.45-19.23	p≤0.01
	Mean ± SD	11.96±0.89	18.07±0.87	
	% of change	-----	51.09±5.67	
Mean corpuscular Volume MCV (fL)	Range	73.54-89.90	54.67-65.11	p≤0.01
	Mean ± SD	81.20±5.10	61.66±4.22	
	% of change	------	-24.06±4.87	
Reticulocyte hemoglobin content (RHC, pg)	Range	30.67-34.93	26.15-28.06	p≤0.01
	Mean ± SD	31.93±1.19	27.14±1.67	
	% of change	------	-15.00±2.54	

Table 3. Biochemical markers of IDA and healthy controls groups

Parameters		Normal group (n=150)	IDA group (n=91)	Statistical analysis
Serum iron (µg/dl)	Range	122.45-154.89	29.78-35.35	p≤0.001
	Mean ± SD	131.11±20.90	31.10±3.02	
	% of change	-----	-76.28±6.45	
Serum ferritin (µg/dl)	Range	107.21-140.34	7.01-8.90	p≤0.001
	Mean ± SD	122.12±15.65	7.50±3.89	
	% of change	------	-93.86±8.11	
Zinc protoporphyrin /heme (µmol/mol)	Range	31.34-38.15	68.36-78.45	
	Mean ± SD	35.99±4.21	72.89±5.11	
	% of change	-------	102.53±10.45	
Transferrin saturation (Tf sat, %)	Range	34.56 – 43.67	7.21-9.18	p≤0.001
	Mean ± SD	38.45±10.56	7.99±2.78	
	% of change	-------	-79.22±6.45	

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Table 4. RBC's glutathione fractions of IDA and healthy controls groups

Parameters		Normal group (n=29)	IDA group (n=29)	Statistical analysis
Reduced glutathione (GSH, µmol/L)	Range	8.08-11.32	5.04-6.78	p≤0.01
	Mean ± SD	9.06±2.05	5.01±1.99	
	% of change	------	-44.70±8.11	
Oxidized glutathione (GSSG, µmol/L)	Range	0.60-0.91	0.51-0.60	p≤0.05
	Mean ± SD	0.73±0.31	0.58±0.17	
	% of change	------	-20.55±2.67	
GSH/GSSG Ratio	Range	11.02-14.04	7.45-9.85	p≤0.01
	Mean ± SD	12.41±0.99	8.63±0.87	
	% of change	------	-30.46±1.90	

Table 5. RBC's antioxidant enzyme activities of IDA and healthy control groups

Enzyme		Normal group (n=29)	IDA group (n=29)	Statistical analysis
Glutathione peroxidase, (GSH-Px, U/g Hb)	Range	13.83-22.76	10.07-18.32	p≤0.01
	Mean ± SD	16.79±2.11	13.09±3.54	
	% of change	------	-22.04±3.67	
Glutathione reductase (GSH-Rd, U/g Hb)	Range	9.99-19.54	8.51-17.84	p≤0.05
	Mean ± SD	14.01±1.32	11.69±2.05	
	% of change	------	-16.59±3.11	
Superoxide dismutase (SOD, U/g Hb)	Range	2.88-45.96	1.23-3.71	p≤0.05
	Mean ± SD	3.85±0.69	2.61± 0.47	
	% of change	------	-32.21±8.03	
Catalase (CAT, U/g Hb)	Range	141.01-180.55	109.53-140.77	p≤0.05
	Mean ± SD	157.28±19.76	115.86±21.62	
	% of change	------	-26.34±4.67	

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Table 6. Plasma antioxidant vitamins level of IDA and healthy control groups

Vitamins		Normal group (n=29)	IDA group (n=29)	Statistical analysis
Vitamin A (μmol/L)	Range	1.02-2.33	0.80-0.98	p≤0.05
	Mean ± SD	1.45±0.37	0.85±0.11	
	% of change	------	-41.37±3.67	
Vitamin C (μmol/L)	Range	41.76-55.86	32.90-42.80	p≤0.05
	Mean ± SD	50.54±9.11	38.0±7.54	
	% of change	------	-24.81±5.10	
Vitamin E (μmol/L)	Range	26.76-40.32	19.70-27.15	p≤0.05
	Mean ± SD	31.78±7.15	23.45±8.10	
	% of change	------	-26.21±6.14	

Table 7. Blood levels of oxidants in IDA and healthy controls groups

Oxidants		Normal group (n=29)	IDA group (n=29)	Statistical analysis
Thiobarbituric acid reactive substances (TBARS, nmol/mL)	Range	1.69-2.09	1.98-3.87	p<0.01
	Mean ± SD	1.87±0.39	2.31±0.73	
	% of change	------	23.52±4.10	
Nitrite (NO$_2$, (nmol/L)	Range	1.52-2.36	2.51-2.83	p<0.01
	Mean ± SD	2.01±0.11	2.61±0.22	
	% of change	------	29.85±5.50	
Nitrite/nitrate (NO$_3$/ NO$_2$, nmol/L)	Range	3.10-3.70	3.87-4.28	p<0.01
	Mean ± SD	3.26±0.51	3.97±0.67	
	% of change		21.78±7.90	

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Table 8. Correlation between serum iron, serum ferritin, GSH, TBARS, NO_2 and Hb of IDA patients

Parameter	Correlation equation	r^2
Serum iron/Hb (n=91)	Serum iron (µg/dl) = 7.1412x (Hb, g/dl) - 18.446	0.8523[***]
Serum ferritin/Hb (n=91)	Serum ferritin (%) = 3.7786 (Hb, g/dl) - 11.926	0.8874[***]
GSH/Hb (n=29)	GSH (µmol/L) = 0.8868 (Hb, g/dl) - 2.0257	0.8150[**]
TBARS/Hb (n=29)	TBARS (nmol/mL) = -0.504x (Hb, g/dl) - 6.9513	- 0.6865[**]
NO_2/Hb (n=29)	NO_2 (nmol/mL) = -0.4321x (Hb, g/dl) - 7.4096	- 0.7517[*]

*** significant ($P \leq 0.001$) , ** significant ($P \leq 0.01$), * significant ($P \leq 0.05$)

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دور الحالة المؤكسدة/المضادة للأكسدة في الاصابة بأنيميا نقص الحديد: دراسة على مجموعة أطفال من محافظات القليوبية والمنوفية، مصر

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الملخص العربي

انيما نقص الحديد (IDA) تعنى فقر الدم الناجم عن نقص الحديد، والتى تعد أحد الأسباب الرئيسية للاصابة بالمرض والوفيات في جميع أنحاء العالم والتي تؤثر على افراد المجتمع من جميع الأعمار في كل من البلدان المتقدمة والنامية على السواء. وتهدف الدراسة الحالية إلى استكشاف دور الحالة المؤكسدة / المضادة للأكسدة في الاصابة بأنيميا نقص الحديد وذلك من خلال الدراسة على مجموعة أطفال من محافظات القليوبية والمنوفية بمصر. حيث تم اختيار عدد ٩١ مريضا، ١٥٠ من الأصحاء ، الذكور والإناث، من الرضع والأطفال صغار السن الذين تتراوح أعمارهم بين يوم الولادة و ٦ سنوات من الوحدات الريفية الصحية ومراكز رعاية الأمومة والطفولة بمحافظات القليوبية والمنوفية بمصر لإجراء الدراسة. وقد تم اجراء تحاليل هيماتولوجية وكيموحيوية للمشاركين فى الدراسة. واشارت النتائج أن متوسط مستوى الهيموجلوبين (Hb) ، ومتوسط حجم كرات الدم الحمراء (MCV) ، ومحتوى الهيموجلوبين الشبكي، وحديد وفيريتين الدم للأطفال الطبيعيين كان منخفضة بدرجة معنوية (P≤0.05) مقارنة بالمجموعة الصحية الضابطة. كما سجلت النتائج اتجاها معاكسا بالنسبة لمقياس عرض توزيع خلايا الدم الحمراء (RDW). أما بالنسبة للمقاييس المعبرة عن نظام الدفاع المضاد للأكسدة بما والتى تشمل الإنزيمات (الجلوتاثيون بيرأوكسيديز، الجلوتاثيون ريدأكنيز، ديسموتاز الفائق الأكسدة، والكاتاليز،) وغير الإنزيمات (الجلوتاثيون ، والفيتامينات، ج، هـ) لمرضى انيميا نقص الحديد والتى كانت قيمها أقل انخفاضا بدرجة معنوية° (P≤0.05) بينما كانت مقاييس الإجهاد التأكسدي (المواد النشطة لحمض الثيوباربيتوريك وأكسيد النيتريك) ذات قيم أعلى بدرجة معنوية° (P≤0.05) مقارنة بالمجموعة الصحية الضابطة. وخلصت تلك الدراسة الى أن الحالة المؤكسدة / المضادة للأكسدة يمكن ان تلعب دورا هاما في التسبب في الإصابة بمرض أنيميا نقص الحديد.

الكلمات المفتاحية : المقاييس الهيماتولوجية ، الإنزيمات والفيتامينات المضادة للأكسدة ، وعوامل الاكسده