Evaluation of neonatal jaundice in the Makkah region

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The aims of this study were to detect the frequency at which the different types of neonatal jaundice occur in Makkah and to estimate the malondialdehyde (MDA) levels. This study included 239 neonates with neonatal jaundice, 20 anemic neonates and 21 healthy neonates. ABO incompatibility was observed in 31.6% of neonates with indirect hyperbilirubinemia, in 14.3% of those with early onset jaundice, in 9.5% of those with persistent jaundice, in 8.5% of those with physiological jaundice, in 5% of anemic neonates and in 12% of all neonates. glucose-6-phosphate dehydrogenase (G6PD) deficiency was observed in 10.5% of neonates with indirect hyperbilirubinemia, in 3.9% of those with physiological jaundice, in 11.1% of those with direct hyperbilirubinemia, in 3.9% of those with physiological jaundice, in 11.1% of those with direct hyperbilirubinemia, in 12% of those with persistent jaundice, in 10% of anemic neonates and in 6.6% of all neonates. Rh incompatibility and polycythemia were found in 2.6% of neonates with indirect hyperbilirubinemia and in 0.4% of all neonates. In comparison to control group, MDA was significantly higher in all groups except for the anemic group. In conclusion, ABO incompatibility and G6PD deficiency frequently result in neonatal jaundice in Makkah, whereas Rh incompatibility and polycythemia are rare. The MDA level may serve as an indicator of oxidative stress.

Neonatal jaundice is a benign condition that often does not require intervention. Most cases of neonatal hyperbilirubinemia constitute physiological jaundice and do not have serious consequences. An important clinical challenge is to identify those newborns who are at risk of developing severe neonatal hyperbilirubinemia. There are many factors implicated in the development of pathological jaundice, including perinatal factors (e.g., birth trauma or infections), maternal factors (e.g., Rh or ABO incompatibility), neonatal factors (e.g., prematurity or polycythemia), and genetic factors (e.g., Crigler-Najjar’s or Gilbert’s syndrome). In addition, the administration of drugs such as cephalosporins and glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency have been implicated in pathological jaundice. The mechanism by which hyperbilirubinemia occurs is through either increased bilirubin production (resulting from hemolysis, sepsis, blood extravasation or polycythemia) or increased enterohepatic circulation (resulting from prematurity, pyloric stenosis, delayed bacterial gut colonization, gastrointestinal tract immobility or obstruction), or decreased bilirubin elimination which occurs in Crigler-Najjar’s and Gilbert’s syndromes.

ABO incompatibility usually occurs in the offspring of women with type O blood and occasionally in mothers with both type A blood and high anti-B IgG levels. ABO incompatibility exists in approximately 15–25% of all maternal/fetal pairs. However, ABO hemolytic disease of the newborn occurs in approximately 1% of group O mothers with high antenatal IgG antibody titer. Currently, the use of prophylactic anti-D immunoglobulin greatly reduces the incidence of hemolytic disease of the newborn due to Rh incompatibility.

G6PD deficiency is an X-linked recessive disease that results in clinical manifestations such as neonatal jaundice, chronic nonspherocytic anemia, infection and drug-induced hemolysis. A role for G6PD deficiency in the genesis of neonatal hyperbilirubinemia has been reported. The most serious complication of G6PD deficiency in newborns is kernicterus resulting from severe neonatal hyperbilirubinemia.

Infants who experience chronic or acute fetal hypoxia have a higher incidence of polycythemia, with hematocrit levels greater than 65%. Neonatal hyperbilirubinemia is a common clinical feature.

Hyperbilirubinemia can also result from increased oxidative stress. It has been reported that bilirubin function as a scavenger of reactive oxygen species. Malondialdehyde (MDA) is a reactive metabolic product that results from the effects of reactive oxygen species on tissues and from a series of reactions that occur during lipid peroxidation. Plasma MDA levels are a sensitive indicator of lipid peroxidation and thus of oxidative stress.

Therefore, measuring the MDA concentration in hyperbilirubinemic newborns will help determine the relationship between oxidative stress and elevated bilirubin levels. The aim of this study was to determine the frequency at
which the different types of neonatal jaundice occur in the Makkah region. We also sought to determine the prevalence of ABO incompatibility, Rh incompatibility, G6PD deficiency, and polycythemia in neonates with different types of jaundice. Finally, we sought to estimate the levels of MDA and correlate them with the various parameters studied.

**Results**

This study included 239 jaundiced neonates, 20 anemic babies, and 21 healthy neonates (control group). The neonates consisted of 169 males and 111 females (a ratio of 3 male: 2 female). The numbers of neonates in each group was G1, n = 21, G2, n = 38, G3, n = 129, G4, n = 9, G5, n = 42, G6, n = 21, and G7, n = 20 neonates (Table 1). The total number of neonates with hyperbilirubinemia and anemia was 259. Of these neonates, 74 were preterm. The members of groups 2, 3, and 6 were significantly younger (p < 0.001) than those in the control group (G1). In contrast, those in group 7 were significantly older than those in G1 (Table 2). Six neonates (15.8%) were preterm in G2, 37 (28.7%) were preterm in G3, 1 (11.1%) was preterm in G4, 15 (35.7%) were preterm in G5, 9 (42.9%) were preterm in G6 and 6 (30%) were preterm in G7. No significant differences were found with regard to age among preterm and term infants in any of the groups (p > 0.05).

Several parameters were evaluated for each group. The results are summarized in Table 2. Comparisons between the different groups were performed using the Kruskall-Wallis test. We found highly significant differences between the groups in age, total bilirubin levels, direct bilirubin levels, G6PD activity, red blood cell counts and hemoglobin concentrations (p < 0.001). No significant differences in MDA levels were found (p > 0.05).

As shown in Figure 1, the frequencies of neonatal jaundice are as follows: physiological jaundice-53.9% (129/239), persistent jaundice-17.6% (42/239), indirect hyperbilirubinemia-15.9% (38/239), early onset jaundice-8.8% (21/239), and direct hyperbilirubinemia-3.8% (9/239).

The highest prevalence of ABO incompatibility was found in the indirect hyperbilirubinemia group (12/38, 31.6%) followed by the early onset jaundice group (3/21, 14.3%), the persistent jaundice group (4/42, 9.5%), the physiological jaundice group (11/129, 8.5%) and the anemic group (1/20, 5%). The frequency of ABO incompatibility in all studied groups was 31/259 (12%) (Fig. 2). ABO incompatibility was determined by clinical classification of the mother as type O and the neonate as type A or B.

Regarding hemoglobin concentrations in neonates with ABO incompatibility, no significant differences were observed between the control group and the indirect hyperbilirubinemia group, the physiological jaundice group, or the early onset jaundice group. However, the persistent jaundice group had significantly decreased hemoglobin levels than the control group (11.0 ± 1.4, p = 0.01).

The frequency of Rh incompatibility was 1/38 (2.6%) in the indirect hyperbilirubinemia group. Rh incompatibility occurred in 0.4% of all neonates. The frequency of polycythemia was 2.6% (1/38) in the indirect hyperbilirubinemia group and 0.4% of all neonates. The direct Coombs’ test was used in all cases. A positive result was found in only 5 cases of ABO incompatibility (16.1%) and in only one case of Rh incompatibility.

The frequency of G6PD enzyme deficiency among the different groups is shown in Table 3. The classification of G6PD deficiency was performed according to the WHO17. No cases of class I G6PD deficiency were found. However, 10 cases of class V were observed. Of these, 2 were in G2 (one male and one female), 6 were in G3 (five males and 1 female), 2 were in G5 (both female), and 2 were in G6 (both female). No significant differences were found between males and females regarding G6PD concentrations, with the exception of G6, the early onset of jaundice, which showed significantly higher G6PD concentrations in the female group than in the male group (p > 0.05 and p < 0.05, respectively) (Table 4). There was no significant difference in the concentrations of hemoglobin between the neonates with G6PD deficiency and the control group. The hemoglobin concentrations in each group were 13.7 ± 3.1 and 15.3 ± 2.0, respectively.

Using the Spearman correlation, a significant negative correlation was found between MDA and hemoglobin levels in the persistent jaundice group (r = −0.98, p = 0.01). No other significant correlations were detected.

**Discussion**

Neonatal hyperbilirubinemia is associated with a variety of conditions. Severe NH poses a direct risk of permanent neurological sequelae. The early identification of neonates who are at a greater risk of developing severe neonatal hyperbilirubinemia is of paramount importance to preventing brain damage17. In this study, the type of jaundice found at the highest frequency was physiological jaundice (53.9%) followed by persistent jaundice (17.6%), indirect hyperbilirubinemia, early onset jaundice and direct hyperbilirubinemia (Fig. 1). These findings are in line with various national and international studies16-17. Physiological aspects that can contribute to neonatal hyperbilirubinemia include increased bilirubin production, less efficient hepatic conjugation, and enhanced bilirubin absorption by the enterohepatic circulation18. Bilirubin has been proposed as an effective antioxidant, and modest elevations of bilirubin may be beneficial in neonates19.

ABO incompatibility and G6PD deficiency were found to be frequent causes of neonatal hyperbilirubinemia in our study, as well as in other studies. G6PD deficiency is the most common human genetic enzymopathy, affecting over 200 million individuals worldwide. It is closely associated with neonatal jaundice, chronic nonspherocytic hemolytic anemia, favism and food- or drug-induced acute hemolytic anemia20. In this study, class II G6PD enzyme deficiency

| Table 1 | Classification of the neonates |
|---|---|---|---|
| Groups | Name | n | Male | Female |
| G1 | Normal neonates | 21 | 13 | 8 |
| G2 | Indirect hyperbilirubinemia | 38 | 15 | 23 |
| G3 | Physiological jaundice | 129 | 75 | 54 |
| G4 | Direct hyperbilirubinemia | 9 | 7 | 2 |
| G5 | Persistent jaundice | 42 | 27 | 15 |
| G6 | Early onset jaundice | 21 | 16 | 5 |
| G7 | Anemic group | 20 | 16 | 4 |
| Total number | 280 | 169 | 111 |

Criteria: Normal newborn without jaundice or anemia. ≥ indirect bilirubin 5 mg/dl in 0 h, ≥10 mg/dl in 24–48 h of age, and ≥13 mg/dl in ≥ 48 h of age. Bilirubin high but does not reach the cutoff for indirect hyperbilirubinemia in relation to the age of the subjects. Direct bilirubin >2 mg/dl (>20% of total bilirubin). Persistent high bilirubin >2 w in full term baby; >3 w in preterm baby. Appearance of jaundice at 0 to 24 h of age with indirect bilirubin <5 mg/dl. Anemia without jaundice.
Table 2 | A comparison of the studied clinical and laboratory parameters in all groups

| Groups studied | Age days | Total bilirubin mg/dl | Direct bilirubin mg/dl | Indirect bilirubin mg/dl | Malondialdehyde IU/l | Red blood cells \( \times 10^{12} \)/l | Hemoglobin g/dl |
|---------------|----------|----------------------|------------------------|-------------------------|----------------------|-----------------------------|---------------|
| G1            | Mean ± SD | 17.4 ± 11.1          | 0.7 ± 0.26             | 0.1 ± 0.08              | 0.6 ± 0.2            | 4.0 ± 1.0                   | 4.8 ± 0.7     | 15.5 ± 2.2   |
| Median (range) | 21 (0–30) | 0.85 (0.2–1.0)       | 0.1 (0.0–0.3)          | 0.7 (0.2–0.8)           | 3.7 (3.0–7.5)        | 4.8 (4.0–6.2)               | 15 (13–20)    |
| G2            | Mean ± SD | 5.1 ± 3.9            | 12.6 ± 2.9             | 0.7 ± 1.6               | 12.0 ± 2.7           | 8.0 ± 4.5                   | 4.8 ± 0.8     | 15.8 ± 3.1   |
| Median (range) | 4.5 (0–14) | 13.0 (5–16)           | 0.4 (1–10.1)          | 12.6 (4.8–15.7)***      | 6.0 (3.3–18.3)***    | 4.8 (3.3–6.2)              | 16.3 (10.4–21.6) |
| G3            | Mean ± SD | 5.9 ± 3.5            | 7.2 ± 2.9              | 0.4 ± 0.3              | 6.2 ± 2.9            | 7.8 ± 5.1                   | 4.1 ± 0.8     | 14.7 ± 2.8   |
| Median (range) | 5 (2–14) | 7.3 (1–12.5)***      | 0.3 (1–12.5)***        | 6.4 (0–12.5)***         | 6.7 (2–3.8)***       | 6.4 (2–5.6)***             | 14.7 (8–29)   |
| G4            | Mean ± SD | 24.3 ± 6.7           | 6.9 ± 6.3              | 4.9 ± 3.4             | 2.0 ± 2.9            | 10.9 ± 2.9                  | 3.8 ± 0.9     | 10.0 ± 2.4   |
| Median (range) | 28 (14–30) | 5.0 (3–13.6)***      | 3.9 (2–10)***          | 0.9 (0.2–4.9)***        | 11.0 (8–17)***       | 4.2 (2–5.6)***             | 10.0 (7–13–0)*** |
| G5            | Mean ± SD | 20.8 ± 4.6           | 3.5 ± 2.7              | 0.7 ± 0.5             | 2.9 ± 2.8            | 9.0 ± 5.5                   | 3.9 ± 0.7     | 11.9 ± 2.0   |
| Median (range) | 21 (14–30) | 2.5 (0–13)***        | 0.6 (0–10)***          | 1.8 (0–10)***          | 7.6 (3–19)***        | 3.8 (2–7.4)***             | 12.0 (8–15)*** |
| G6            | Mean ± SD | 0.8 ± 0.4            | 3.8 ± 2.0              | 0.2 ± 0.1             | 3.5 ± 2.0            | 6.1 ± 0.5                   | 4.7 ± 1.1     | 15.2 ± 2.9   |
| Median (range) | 1 (0–1)*** | 3.0 (1–8)***         | 0.2 (0–1)***           | 2.8 (0–7.5)***         | 6.2 (5.8–6.5)*       | 5.2 (2–6.2)***             | 16.0 (10–21)  |
| G7            | Mean ± SD | 24.4 ± 11.4          | 0.53 ± 0.33            | 0.1 ± 0.08            | 0.73 ± 0.09          | 7.1 ± 4.6                   | 3.8 ± 0.6     | 11.0 ± 1.7   |
| Median (range) | 24.5 (0–60)* | 0.9 (0–5.1)          | 0.1 (0–0.3)            | 0.8 (0.5–0.9)         | 5.0 (4.3–14)         | 3.9 (2–7.4)***             | 12.0 (8–13)*** |

The values are expressed as the mean ± SD, median, and range. All comparisons were made with control subjects. Significant differences are indicated as * for P < 0.05, ** for P < 0.01, and *** for P < 0.001. Student's t test was used when the data were found to be normally distributed, and the Mann-Whitney U test was used for non-normally distributed data.
reports42,43. These reports showed that a positive direct Coombs’ test in the remaining cases was negative, which is similar to previous findings develop neonatal jaundice22,37. Moreover, Tanphaichitr et al. Previous studies reported that one-third of children with G6PD deficiency have higher frequency of jaundice in neonates with G6PD deficiency. Our results indicate a frequency of G6PD deficiency in all newborns, the positive predictive value was found to be as low as 23% with readmission rates that were similar irrespective of the groups. By contrast, studies from China34, Nigeria35, and Thailand36 found higher frequencies of G6PD deficiency, at 18.42%, 25.5%, and 38%, respectively. In our study, 88.2% (15/17) of G6PD-deficient patients developed neonatal jaundice, of which 33.3% (5/15) were in the physiological jaundice group and the remaining cases had other types of jaundice. Our results indicate a higher frequency of jaundice in neonates with G6PD deficiency. Previous studies reported that one-third of children with G6PD deficiency develop neonatal jaundice23,37. Moreover, Tanphaichitr et al. (1995) reported that 49% of G6PD patients developed neonatal jaundice, of which 28.82% were classified as physiological and 20% as pathological38. The prevalence of the different subtypes of jaundice could explain the different presentations in different geographical locations worldwide.

Neonates with blood group A or B born to group O mothers are defined as having ABO incompatibility39. In our study, out of 259 neonates, 12% were ABO incompatible, which is in agreement with the national data28 but is lower than results of international studies30,31. The direct Coombs’ test was positive in only 16.1% cases, and the remaining cases were negative, which is similar to previous reports32,43. These reports showed that a positive direct Coombs’ test in ABO incompatible neonates increases the risk of significant hyperbilirubinemia; however, when evaluated as a screening test for all newborns, the positive predictive value was found to be as low as 23% with readmission rates that were similar irrespective of the results from the Coombs’ test in ABO-incompatible children.

In most cases, ABO hemolytic disease of newborns causes hyperbilirubinemia without severe neonatal anemia, which is explained by the relatively few group A or B antigens on neonatal red blood cells and the presence of A and B antigens on other tissues and in body fluids44. This result was confirmed by our study, as most ABO incompatible cases had normal hemoglobin levels. Additionally, in our study, ABO incompatibility and G6PD were common causes of neonatal jaundice. If these are present, either phototherapy or intravenous immunoglobulin or both are administered to prevent severe jaundice and to decrease the need for exchange transfusion. Phototherapy was initiated based on the American Academy of Pediatrics phototherapy chart, whereas intravenous immunoglobulin was administered in ABO cases if the total bilirubin was two lines below the exchange transfusion line, in accordance with the exchange transfusion chart.

In the indirect hyperbilirubinemia group, one neonate presented with Rh hemolytic disease of the newborn (a frequency of 2.6% in the indirect hyperbilirubinemia group and 0.4% of all neonates). This finding is in agreement with previous studies, which state that Rh hemolytic disease of the newborn is less common than previously owing to the administration of Rh Ig, which results in a greater than 90% reduction in the alloimmunization rate among treated women45,46.

In this study, one neonate in the indirect hyperbilirubinemia group was polycythemic (2.6%). Although polycythemia can reflect normal fetal adaptation, it has been thought to be responsible for abnormalities in neonates47.

MDA levels are a sensitive indicator of lipid peroxidation and thus of oxidative stress. Increased concentrations of free oxygen radicals in newborns damage the cell membrane through lipid peroxidation, and this damage may be associated with various pathologies such as hypoxic ischemic encephalopathy, intraventricular hemorrhage, necrotizing enterocolitis, and bronchopulmonary dysplasia48. Bilirubin is an effective scavenger of oxidant radicals, and its concentration is increased during oxidative stress. In this study, the MDA concentrations were significantly higher in all of the neonates with the various types of jaundice than in the control group (Table 2). In agreement with our results, Yigit et al (1999) found MDA concentrations in infants with hyperbilirubinemia to be remarkably high44. These MDA levels were thought to result from the response of the neonates to oxidative stress. It has been suggested that hyperbilirubinemia might play a defensive role against oxidative stress because the body’s antioxidant mechanisms are not yet fully developed. It is known that biliary pigment can protect the cell membrane from lipid peroxidation49. In addition, some neonates in our study had infections, perinatal asphyxia, and respiratory distress syndrome, and approximately 30% of our cases were preterm. All of these conditions are considered to contribute to the elevation of MDA concentrations46-51.

In contrast to our results, Kumar et al (2007) found lower MDA levels in neonates with jaundice. This finding may arise from their study being conducted in neonates without high bilirubin levels52. At higher bilirubin levels, the antioxidant effects of bilirubin might be

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### Table 3 | The frequency of G6PD enzyme deficiency in each group

| Groups | Males | Females | Males | Females | Total |
|--------|-------|---------|-------|---------|-------|
| G2     | 38    | 1/38 (2.6%) | –     | –       | 3/38 (7.9%) | 4/38 (10.5%) |
| G3     | 129   | 1/129 (0.8%) | –     | –       | 4/129 (3.1%) | 5/129 (3.9%) |
| G4     | 9     | –         | 1/9 (11.1%) | –       | 1/9 (11.1%) | 5/42 (12.0%) |
| G5     | 42    | 2/42 (4.7%) | –     | 3/42 (7.1%) | 2/42 (10%)  | 2/42 (10%)  |
| G6     | 21    | –         | –     | –       | –     | –     |
| G7     | 20    | –         | –     | 2/20 (10%) | 2/20 (10%) | 9     |
| Total  | 259   | 4         | –     | 259     | 9     | 17/259 (6.6%) |

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### Table 4 | A comparison between males and females with regard to G6PD concentration (U/gHb) among the different groups

| Groups | Males Mean ± SD | Females Mean ± SD | P-value |
|--------|----------------|-------------------|---------|
| G1     | 12.0 ± 2.5     | 12.4 ± 2.4        | 0.828   |
| G2     | 14.5 ± 6.6     | 11.8 ± 4.2        | 0.203   |
| G3     | 13.7 ± 4.8     | 12.3 ± 4.1        | 0.192   |
| G4     | 9.5 ± 4.0      | 12.7 ± 3.1        | 0.498   |
| G5     | 8.8 ± 4.2      | 13.8 ± 6.8        | 0.057   |
| G6     | 13.9 ± 2.9     | 20.7 ± 0.9        | 0.013   |
| G7     | 11.2 ± 1.6     | 9.6 ± 4.4         | 0.578   |

Student’s t test was used.

The Mann-Whitney U test was used in these groups.
negated by its direct cellular toxicity. In addition, Kumar et al.
excluded preterm neonates as well as those with infections, perinatal
asphyxia, respiratory distress or major malformation, which were all
included in our study. Furthermore, we found no significant correla-
tion between bilirubin and MDA levels among the studied groups.
This may indicate that factors other than MDA levels may contribute
to the increased oxidative stress in our cases, such as stress and
prematurity. Our results confirm those of Yigit et al. (1998), who
did not find a correlation between MDA levels and bilirubin con-
centrations in infants with non-hemolytic hyperbilirubinemia16.
Moreover, we found a significant negative correlation between MDA and hemoglobin levels in the group with persistent jaundice.
This may indicate that anemia accompanies many pathological pro-
cesses in neonates and can negatively affect prognosis. Thus,
increased MDA levels may indicate oxidative stress14.

One limitation of this study is that some factors that may have led
to neonatal hyperbilirubinemia were not examined such as sphero-
cytosis, thalassemia and sickle cell anemia.

Conclusions and recommendation. ABO incompatibility and
G6PD deficiency are frequent causes of neonatal jaundice in
Makkah, whereas Rh incompatibility and polycythemia are rarely
observed. The MDA level could serve as an indicator of oxidative
stress. Determinations of ABO and Rh incompatibility, G6PD
deficiency and complete blood counts at birth are recommended to
avoid serious complications.

Methods

Subjects. The protocol for this study was approved by the Umm Al-Qura University
ethics committee. All participants gave informed consent in accordance with the
declaration of Helsinki. The analytical methods were carried out in the research
laboratory of the Biochemistry Department, Faculty of Medicine, Umm Al-Qura
University in accordance with the approved guidelines.

This study was carried out from January 2011 to January 2013. All neonates were
attended to at Alnosur Hospital, Makkah, Saudi Arabia. The description and clas-
sification of all subjects is summarized in Table 1, with healthy neonates serving as a
control group (G1). The jaundiced neonates were divided into 5 groups according to
their indirect and direct bilirubin levels, the time of jaundice onset, and the persist-
ence of jaundice17. Members of the indirect hyperbilirubinemia group (G2) were
characterized as having increased levels of indirect bilirubin, where the concentration of
indirect bilirubin was found to be ≥5 mg/dl at 0 h of age, ≥10 mg/dl at 24–48 h of age
and ≥13 mg/dl at more than 48 h of age. In the physiological jaundice group (G3),
the indirect bilirubin levels were not high enough to allow stratification based
on age. In the direct hyperbilirubinemia group (G4), the levels of direct bilirubin were
determined to be ≥2 mg/dl (>20% of total bilirubin). Group 5 (G5) was composed of
neonates with high bilirubin levels that had persistent jaundice for ≥2 weeks in full
term neonates and ≥3 weeks in preterm neonates. Group 6 was composed of neo-
nates with early onset jaundice (G6), in which jaundice was observed in the first 24 h of
life but indirect bilirubin remained <5 mg. Neonates in the anemic group (G7)
were free of jaundice.

Sample collection. Four ml of blood was collected from neonates at the time of
admission under aseptic conditions. 2 ml of this blood was centrifuged for 15
minutes, and the serum was separated to allow MDA and total/direct bilirubin
measurements. The remaining 2 ml of blood was collected in EDTA tubes and used
to perform blood counts, blood group analyses, G6PD enzyme assays, and direct
antiglobulin tests. In addition, 1 ml of blood was taken from each mother for blood
group determination.

All participants were subjected to a:

1. Full clinical history.
2. Complete hemogram analysis using a Sysmex XT 2000i hematology analyzer
(Sysmex Corporation of America, Long Grove, Illinois, USA), including red
blood cells count, hemoglobin, packed cell volume, mean corpuscular
hemoglobin, mean cell volume, mean corpuscular hemoglobin concentration, red
cell distribution width, white blood cells count and platelet count.
3. ABO grouping of baby and mother (Dialab, Austria).
4. Direct antiglobulin test (Coombs’ test) tube method. The reagent was supplied
by Core Diagnostics LTD, Birmingham - United Kingdom.
5. Quantitative measurement of G6PD enzyme levels by the ultraviolet kinetic
method by TurqBioch, USA18.
6. MDA enzyme assay with the VISSelect TRASS Assay Kit from Cell Biolabs,
Inc. (USA)19.
7. Determination of direct and total bilirubin levels using the Dimension Rxl
max integrated chemistry system (Dade Behring, USA)20.

Statistical analyses. All statistical analyses in this study were performed using the
SPSS program (version 16). All quantitative data are described as the mean ± SD, the
median, or the range. Comparisons between the control group and all other groups
were performed using the Student’s t test or the Mann-Whitney U test, depending on
the distribution of the data. The Kruskal-Wallis H test was used when comparing all
groups, as some of the data were not normally distributed. In addition, the Spearman
correlation was used when correlating MDA with other parameters studied. Statistical
significance was defined as p < 0.05.
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Author contributions
A.E.: Design of the study, clinical selection, diagnosis and classification of the cases, writing and revising of the main manuscript text. E.E.N.E.: Design of the study, performance of biochemical laboratory investigations, preparing tables and figures, editing, styling, writing and revising of the main manuscript text. A.Z.: Performance of hematological laboratory investigations, statistical analysis, writing and revising of the main manuscript text. S.M.: Performance of hematological laboratory investigations, writing and revising of the main manuscript text.

Additional information
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