

Relationship of glucose-6-phosphate dehydrogenase deficiency and neonatal sepsis: a single-center investigation on the major cause of neonatal morbidity and mortality

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Introduction: Neonatal sepsis is a serious disease with distinct clinical and laboratory findings. G6PD deficiency is known as the most common human erythrocyte-enzyme deficiency. This study was designed to investigate the relationship between G6PD deficiency and neonatal sepsis, since it is a major cause of neonatal morbidity and mortality.

Methods: A cross-sectional case-control study was designed and performed on 50 neonates who had been admitted to the neonatal intensive-care unit and diagnosed with sepsis and 50 normal neonate controls. Quantitative G6PD-enzyme activity was assessed in the case and control groups.

Results: Quantitative G6PD-level assessment showed that five (5%) subjects in the case group vs one (1%) of the control group were severely deficient and nine (9%) cases vs one (1%) control were moderately deficient. Enzyme-level differences were statistically significant ($P=0.003$).

Conclusion: Our study showed higher incidence of G6PD deficiency in neonates who had been admitted due to sepsis. We suggest quantitative G6PD-level assessment instead of the routine qualitative methods in prevalent G6PD deficiency. It is also recommended that neonates with G6PD deficiency be under close supervision during the first month of life, especially those with other risks of neonatal sepsis, such as prematurity or low birth weight.

Keywords: G6PD, neonatal sepsis, morbidity

Introduction

Neonatal sepsis is a serious disease with defined clinical and laboratory findings, which is confirmed through positive blood culture. If is not diagnosed rapidly and treated completely, it can lead to permanent disability, multiple organ failure, shock or death.^{1,2}

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is present in more than 400 million people worldwide as the most common erythrocyte human enzyme deficiency, with the highest prevalence in Asia, Africa, the Mediterranean and the Middle East.³ According to the world health organization (WHO), the prevalence of G6PD deficiency is 10–14.9% in Iran. A study on the prevalence of G6PD deficiency in Fars province (southern Iran) showed that 1.8% of all females and 12% of males had G6PD deficiency.^{4,5,17}

The exact mechanism for higher prevalence of sepsis in male neonates is not clear.¹⁸ This x-linked recessive enzyme deficiency presents in a spectrum of clinical

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manifestations such as infection, neonatal jaundice, chronic anemia, and drug induced hemolysis.⁷ Also, G6PD enzyme plays a major role in glucose metabolism, and its presence is vital for nicotinamide adenine dinucleotide phosphate (NADPH) production. One of the NADPH functions is glutathione reduction, which detoxifies and neutralizes the oxidants.

This hypothesis proposes that G6PD deficiency significantly reduces the NADPH content of white blood cells (WBCs); hence, it negatively affects the fighting ability against invasive bacterial infection.^{8–12} Few studies have shown the relationship between G6PD deficiency and increase in the incidence of some infections like viral hepatitis, post-operation infection and sepsis.⁶ Since neonatal sepsis is a major cause of neonatal morbidity and mortality, this study was designed to investigate the relationship between G6PD deficiency and neonatal sepsis.

Material and methods

A cross-sectional case control study was designed and performed on 50 neonates who were admitted to neonatal intensive care unit (NICU) diagnosed with sepsis in Zeynabieh hospital affiliated to Shiraz University of Medical Sciences from January 2017 to January 2018. Also, 50 healthy controls (neonate who referred to Zeynabieh clinic for a routine check up and vaccination) were included in this study. Written informed consent was obtained from all the patients and control's parents/guardians. The Ethics Committee of Jahrom University of Medical Sciences approved the study with the code number of Jums.REC.1393.129.

Before the administration of antibiotic, blood sample site was prepared according to the CLSI guidelines. Then, the whole blood sample was taken from all the neonates (1cc) in EDTA tube, and transferred to Neshate laboratory complex stored in cool box at 2–8 °C. Red blood cells' G6PD enzyme level was quantitatively measured by Hitachi 911 full-automatic device (Weber company kit, Japan). Enzyme activity was determined by the international unit per gram of haemoglobin (IU/gHb). In this method, the normal enzyme activity reference range was ≥ 6.5 IU/gHb. Enzyme activity levels between 1.5 and 6.5 IU/gHb were considered as intermediate deficiency and below 1.5 IU/gHb as severe deficiency.

The inclusion criteria were neonates who had been admitted to NICU with C-reactive protein level greater than 6 mg/lit, $5,000 \leq \text{WBC} \leq 25,000$ (mcl), immature neutrophils/total neutrophils greater than 0.2, and positive or negative blood culture. The exclusion criteria were rejection

of the patients' guardian for taking blood sample and patients who were admitted to NICU with different diagnosis such as respiratory distress, metabolic disorders and prematurity.

Data analysis

Data analysis was performed using SPSS, version 20. Comparison of qualitative variables between the groups was done using Chi-square test, and the comparison of the quantitative variables between the two groups was done by Student's *t*-test. *P*-values < 0.05 were considered to be statistically significant.

Result

This study was performed on fifty NICU admitted neonates as the case group (27(54%) male and 23(46%) female) and fifty normal neonates as the control group (20(40%) cases male and 30(60%) female). The results of *t*-test for comparing the mean age and Apgar score of the neonates showed that the relationship between the two groups was not significant (Table 1). Mann-Whitney U test results showed no significant difference between the mean values of hemoglobin in both groups (Table 1). The results of Chi-square test showed that the association between the two groups regarding gender was not significant (Table 1). The mean hemoglobin levels in the case and control groups were 14.3 ± 2.75 and 13.9 ± 2.84 , respectively, which was not statistically significant. The first minute Apgar score was not statistically significant between the two groups. Gender distributions of G6PD deficiency was statistically significant (Table 2).

quantitative G6PD enzyme level assessment in the case and control groups showed that 5 (5%) of the cases vs 1 (1%) of the controls were severely deficient (G6PD enzyme level < 1.5), 9 (9%) of the cases vs 1 (1%) of the controls were moderately deficient (G6PD enzyme level: 1.5–6.5), and 36 (36%) of the cases vs 48 (48%) of the controls were normal (G6PD enzyme level > 6.5). There was a higher rate of G6PD deficiency in the sepsis group than the control group ($P = 0.003$). Regarding the non-match of SEX with group factor at the beginning of the study (according to Table 1), the relationship between G6PD and group with a control on sex should be monitored.

Table 2 showed that although all six people who were G6PD deficient were male and that the relationship between G6PD and group was not significant in men ($p = 0.09$), but the relation between G6PD and group in female is significant ($p = 0.04$).

Table 1 Demographic and clinical data of the two groups: normal and sepsis

Demographic factor		Sepsis group, N=50	Normal group, N=50	Total	Statistical index	P-value (confidence interval)
Age (days)	Mean \pm SD	8.96 \pm 4.15	10.52 \pm 3.98	9.78 \pm 4.15	1.93 (t-test)	0.057 Sepsis: (95% CI: 7.79-10.13) Normal: (95% CI: 9.39-11.65)
Sex, n (%)	Male Female	27(27%) 23(23%)	(% 20) 20 (% 30) 30	(% 47) 47 (% 53) 53	1.97 (Chi-square)	0.161
Apgar	Mean \pm SD	8.12 \pm 1.08	8.36 \pm 0.88	8.24 \pm 0.99	1.22 (t-test)	0.225 Sepsis: (95% CI: 7.81-8.43) Normal: (95% CI: 8.11-8.61)
Hemoglobin (g/dL)	Mean \pm SD	14.37 \pm 2.76	13.99 \pm 2.85	14.18 \pm 2.79	1097.5 (Mann-Whitney U test)	0.246 Sepsis: (95% CI: 13.18-14.8) Normal: (95% CI: 13.59-15.15)

Table 2 Association of G6PD enzyme level between the two groups (sepsis and normal)

Sex	G6pd (units/gram)	Sepsis group	Normal group	Total	OR (95% ci for OR)	Statistics	P-value
Male	Deficient (<1.5)	5 (10.6)	1 (2.1)	6 (12.8)	5.63 (0.59-53.4)	5.04 (Fishers exact)	0.09
	Intermediate (1.5-6.5)	6 (12.8)	1 (2.1)	7 (14.9)	6.75 (0.73-62.2)		
	Normal (\geq 6.5)	16 (34)	18 (38.3)	34 (72.3)	1		
Female	Deficient (<1.5)	0 (0)	0 (0)	0 (0)	-	4.15 (Fishers exact)	0.04
	Intermediate (1.5-6.5)	3 (5.7)	0 (0)	3 (5.7)	-		
	Normal (\geq 6.5)	20 (37.7)	30 (56.6)	50 (94.3)	1		

Discussion

G6PD enzyme deficiency, as the most common human enzyme deficiency is an X-linked recessive disease. G6PD deficiency may present itself as an acute hemolytic anemia and neonatal jaundice after ingestion of fava beans (favism) and some drugs.^{13,14}

The enzyme deficiency is related to some degrees of immune deficiency, but few studies have been conducted on the relationship between enzyme deficiency and incidence of neonatal sepsis development. The exact mechanism that can explain the relationship of neonatal sepsis incidence with G6PD deficiency is still unknown. One of the hypotheses states reactive oxygen species synthesis reduction due to

NADPH shortage affects the immune system. However, due to G6PD deficiency, produced NADPH is not enough to neutralize all the oxidants produced. Consequently, these oxidants cause damage and apoptosis of the neutrophils.¹⁰

Another study states that increased risk of sepsis is due to serum iron level increment related to hemolysis. For example, Oppenheimer reported that intramuscular injection of iron increased the prevalence of sepsis in the meningitis patients.¹⁵ For more accurate study of G6PD enzyme level, we evaluated it qualitatively, but we took it into account quantitatively.

Our study showed a higher incidence of G6PD deficiency in neonates who were admitted with impression of

sepsis. Ardati et al concluded in their research that the reduced activity of G6PD to as low as 23% of the normal does not affect the neutrophil function.⁶

Cooper et al reported that G6PD deficiency increased the overall chance of infection likewise,¹⁶ G6PD deficiency did not increase the neonatal sepsis risk in a study by Zareifar et al,¹⁷ but Rostami-Far et al's study revealed a higher prevalence of deficiency in the patient group in comparison with the controls.¹⁸ Some of the limitations of this study were: Parental dissatisfaction for participating in the study and taking blood samples, High cost of doing laboratory tests and the low rate of G6PD deficiency in the control group. We were able to confirm higher G6PD deficiency frequency in septic neonates than control group. Also, we can take G6PD deficient neonate into consideration at risk of sepsis, but true risk assessment of G6PD deficiency with neonatal sepsis requires a multi-center cohort study, which into account takes the duration of admission, suspicions to sepsis and confirmed morbidity and mortality due to sepsis into account. Although the association between G6PD and group was not significant in men (0.09) but it was close to nominal level (0.05) and if the sample size is higher, the design is more stable and the association of G6PD and group could be significant in male.

We also suggest quantitative G6PD level assessment instead of routine qualitative methods in prevalent G6PD deficiency regions because the quantitative test is more sensitive and can show the severity of enzyme deficiency (normal, deficient and intermediate) and can identify heterozygotes that were not detected using the spectrophotometric G6PD assay alone.¹⁹

Also, it is recommended that the deficient neonates should be under close supervision during the first month of life, especially those with other risks of neonatal sepsis like prematurity or low birth weight.

Ethical approval

This study was approved by the local Ethics Committee of Jahrom University of Medical Sciences (code number of Jums.REC.1393.129) and written informed consent was obtained from a parent for each of the participants. This study was conducted in accordance with the Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest in this work.

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