G6PD Deficiency in Palestinian children increases risk of hospitalization which differs according to G6PD variant

N. Scott Reading[1], Mahmoud Sirdah[2], Sherrie L. Perkins[1,3], Andrew R. Wilson[1], Mohammad E. Shubair[4], Lina Aboud[5] and Josef T. Prchal[1,3,6]

[1]ARUP Laboratories ,Salt Lake City, UT - USA; [2]Biology Department, Al Azhar University-Gaza, Palestine; [3]Department of Pathology, University of Utah, Salt Lake City, UT - USA; [4]Department of Laboratory Medical Sciences, Islamic University-Gaza; [5]Al Nasser Pediatric Hospital, Palestinian Ministry of Health, Palestine; [6]Division of Hematology and Hematologic Malignancies, Department of Internal Medicine, University of Utah, Salt Lake City, UT - USA

ABSTRACT:

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, affecting more than 500 million people worldwide, is one of the most common inherited red blood cell disorders. There are 186 G6PD mutations published, with some that cluster within defined ethnic/racial groups. These prevalent variants are generally considered to be benign in adults, but their morbidity, presenting as acute hemolytic anemia in young children, can be significant. However, comprehensive molecular characterization of ethnically prevalent G6PD mutants and their relative clinical significance is lacking. In the Gaza Strip, where the G6PD deficiency frequency in the population is significant (>3%) and fava beans, green and dried, are a dietary staple, the risk of acute hemolytic crisis is significant. We studied 80 unrelated Gaza Strip Palestinian children (ages 34–52 months, mean=39.5) hospitalized for acute hemolytic anemia. We found a dramatically increased risk of hospitalization for acute hemolytic anemia among Palestinian children due to G6PD deficiency. The risk varied in accordance with the G6PD variant associated with the disorder. This is the first study comparing the relative clinical impact of three prevalent G6PD deficient variants in the same population and habitat to identify clinically significant effects due to the different genetic mutations.

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MATERIALS & METHODS:

Assay Design

IRB and Consent: The study was approved by the Helsinki Ethical Committee at the Palestinian Ministry of Health. Informed consent was obtained from adults and the parents or guardians of children enrolled in the study. **Sample Type/Source:** Whole blood, Genomic DNA (gDNA). **Methodology:** CBC, G6PD enzyme assay, Polymerase Chain Reaction (PCR), Sequencing, Single Nucleotide Extension (SNE). **Design: Clinical Population:** 80 Palestinian children hospitalized for hemolytic anemia. **Control Population:** 40 healthy Palestinian children (CBC,G6PD enzyme activity); 466 healthy Palestinian adults (*G6PD* genetic analysis). **G6PD Enzyme Assay:** Randox Laboratories (Antrim, UK) G6PDH Screening Test (U/g Hb at 37ºC). **Polymerase Chain Reaction:** Touchdown PCR (tdPCR) was used to amplify G6PD exons 2-13 which were used for sequencing or SNE analysis. **Sequencing:** Conducted using Big-Dye chemistry 3.1 (Applied Biosystems, Foster City, CA) reference sequence for *G6PD* (NG_009015.1) Analyzed using Mutation Surveyor software (SoftGenetics, LLC, State College, PA). **Single Nucleotide Extension:** ABI Prism SNaPshot Multiplex System using 14 G6PD polymorphisms commonly found in Arab, Southern European, and African groups. **Statistical Analysis:** Logistic regression was used to calculate odds ratios for hospitalization by G6PD variant. Additionally, Fisher's Exact Test was used to determine associations between variant and hospitalization. All analysis was performed using SAS software, ver. 9.3 (SAS Institute Inc., Cary, NC). Results were considered statistically significant if p<0.05.

Table 1: Common G6PD variants comprising SNE assay

Name	Mutation	Name	Mutation	Name	Mutation
Orissa	c.131G	Cairo	c.404C	Kerala	c.949A
Aures	c.143C	*Santa Maria	c.542T	*Betica	c.968C
*A-	c.202A	Mediterranean (MED)	c. 563T	Chatham	c.1003A
Namuro	c.208C	*Mexico City	c.680A	Iowa	c.1156G
A+	c.376G	Seattle	c.844C		

* Mutations often found in association with c.376G mutation.

RESULTS:

Table 2: Summary of demographic and CBC data for male participants and controls.

		G6PD MED	G6PD A-	G6PD Cairo	Normal Controls	Reference ranges
# patients (male)		21	14	11	29	–
Rural residence		10	7	3	–	–
Urban residence		11	7	8	–	–
Age at hospitalization† (p<0.001)	Rural	38.25	38.17	38.0	–	–
	Urban	39.56	39.38	42.83	–	–
Jaundice at birth		yes	yes	yes	–	–
Anemia after fava bean consumption		yes	yes	yes	–	–
G6PD (IU/g Hb)† (p<0.001)		1.12 1.17	1.36 1.02	0.78 0.53	24.34 13.64	6.97-20.5*
RBC (x10^{12}/L)		3.83 0.85	3.62 1.12	3.71 0.85	3.64 0.84	4.0-4.9‡
Hb (g/L)† (p<0.001)		87.76 19.65	88.14 24.88	90.90 15.04	106.3 24.4	105.0-127.0‡
HCT (%)† (p<0.001)		26.54 6.06	26.89 7.63	27.34 4.33	32.03 7.43	31.7-37.7‡
MCV (fL)		71.30 8.57	72.31 10.47	77.02 9.69	88.40 6.71	72.7-84.3‡
MCH (pg)		24.22 3.22	23.77 3.83	25.08 3.86	29.26 0.55	24.1-28.4‡
MCHC (%)		33.54 1.80	32.84 1.52	33.24 0.54	33.39 0.56	31.9-35.1‡

†Statistical significance determined. *Reference range from manufacturer.
‡ARUP Laboratories reference range.

- Differences in G6PD activity levels between variant types was significant for G6PD Cairo and G6PD A-.
- Hb and HCT values indicate significant anemia among males.
- Differences of age at hospitalization was significant between urban and rural children.

Table 3: Critical blood values of G6PD Cairo deficient children four months after hospitalization

Patient number	Gender	Age (mo)	RBC (x10^{12}/L)	HCT (%)	Retic (%)
5	male	56	2.6	22.0	
18	male	44	2.9	26.1	
51	male	45	4.2	28.1	
59	male	44	3.3	28.1	2.2

- G6PD Cairo deficient children tend to be anemic in the absence of hemolytic triggers
- Reticulocyte % is an indicator of the rate of hemolysis

Table 4: Regional common G6PD variants

Country	Mutation (% relative allele frequency)
Iran	MED(78), A-(1.4), Chatham(15), Cosenza$^{c.1376C}$(6.5)
Iraq	MED(89), A-(7)
Jordan	MED(43), A-(24), Chatham(18), Aures(3.6), Valladolid$^{c.406T}$(7)
Kuwait	MED(73), A-(13), Chatham(8.5), Aures(2.4)
Oman	MED(78), A-(8.7), Chatham(8.7)
Saudi Arabia	MED(67), A-(10), Chatham(2.6), Aures(11)
Israel (non-Arab)	MED(89)
UAE	MED(55), A-(17), Aures(17)
Turkey	MED(82), A-(5.5), Chatham(2)
Tunisia	MED(11), A-(64), Aures(5)
Algeria	MED(23), A-(46), Chatham(1), Cosenza(1), Aures(7)

- G6PD MED and A- are the most common G6PD deficient variants found in the Middle East
- G6PD MED average relative frequency – 66%
- G6PD A- average relative frequency – 8.6%
- G6PD Chatham and Aures are common minor variants
- G6PD Cosenza and Valladolid are rare, high frequency variants
- G6PD Cairo not detected at significant levels among regional populations

Table 5: G6PD variants found in Gaza Strip, Palestine

G6PD Variant	G6PD Deficient Children (65 persons/70 X-chromosomes)					
	Male (60)		Female (5)		Allele Frequency	Relative Allele Frequency
	# person	# X-chr	# person	# X-chr		
A+	0	–	0	–	–	–
A-	14	14	2	2	–	**0.24**
MED	21	21	2	3	–	**0.35**
Cairo	11	11	0	–	–	**0.17**
Beverly Hills	1	1	0	–	–	0.014
Gaza$^{c.536A}$	0	–	1	1	–	0.014
G6PD Variant	General Population (466 persons/510 X-chromosomes)					
	Male (420)		Female (46)		Allele Frequency	Relative Allele Frequency
	# person	# X-chr	# person	# X-chr		
A+	6	6	1	2	0.016	0.47
A-	2	2	2	2	0.0078	**0.23**
MED	0	–	3	5	0.0098	**0.29**
Cairo	0	–	0	–	–	–
Beverly Hills	0	–	0	–	–	–
Gaza	0	–	0	–	–	–

- 65 of 80 (81%) Palestinian children hospitalized for hemolytic anemia found to suffer from G6PD deficiency
- Primary clinical G6PD deficiency causing variants are: MED, A- and Cairo
- Unique G6PD variant heterogeneity
- G6PD Cairo is a rare, high frequency variant
- G6PD Gaza is a new G6PD deficient variant

- 3.3% allele frequency for G6PD variants in the general population
- G6PD MED and A- were detected in the hospitalized cohort equivalent to their presence in the general population
- G6PD Cairo was not observed in the general population

Table 6: Distribution of G6PD variants among hospitalized children vs. general Gaza population.

	G6PD Variant					
	Mediterranean		A-		Cairo	
	+	-	+	-	+	-
Hospitalized children	23	57	16	64	11	69
General population	3	463	4	462	0	466
Odds ratio (95% CI)	**70.0 (20.1–367.4)**		**41.1 (13.1–168.2)**		**<∞**	
Fisher's Exact p-value	<0.0001		<0.0001		<0.0001	

Confidence interval (CI)

- G6PD Cairo joins G6PD MED and A- as clinically significant G6PD deficient variants found in the Middle East
- G6PD MED and A- carry a significant risk of hospitalization to children
- G6PD Cairo hospitalization risk to children is considerably higher that that observed for G6PD MED and A-

CONCLUSIONS:

- G6PD deficiency accounts for the majority of Palestinian children hospitalized for hemolytic anemia
- G6PD MED, A- and Cairo present a high risk of hospitalization to children
- G6PD MED, A- and Cairo are predominant G6PD variants in Gaza Strip, Palestine
- Clinical presentation of G6PD Cairo is disproportionate to its presence in the general population.
- G6PD Cairo deficiency has a more severe phenotype for children compared to G6PD MED and A-
- Risk of hospitalization progressively increases from G6PD A-, MED to Cairo

Disclaimer: There are no relevant conflicts of interest to disclose.