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G6PD-DEFICIENCY IN BULGARIAN POPULATION AND SOME RELATED MEDICAL PROBLEMS[1]

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Summary. G6PD-deficiency is a problem of significant medical and social importance for Bulgarian population. Our own population studies on 20 809 individuals revealed a phenotype frequency of $4.10 \pm 0.27\%$ and 0.0286 gene frequency of the Gd^- gene in the country. Prominent genetic polymorphism of the Gd^- variants exists. We have found 14 variants, of which the Mediterranean one is the most frequent — over 50%. Four new variants of G6PD-deficiency have been reported for the first time.

It was found that G6PD-deficiency in newborns with neonatal hyperbilirubinemia is an ethiological factor in 17.31%.

This genetic deffect affects reproductive processes and vitality of the population, increasing prenatal and neonatal mortality among the descendants of G6PD-deficiency carrier.

We discovered pronounced clinico-genetical polymorphism of G6PD-deficiency manifestated as favism, drug-induced anemias, acute hemolytic anemias with unrecognised provoking factor, chronic anemias, etc.

The high frequency and specific genetic and clinical polymorphism of G6PD-deficiency emphasizes an important practical significance of the obtained data.

Glucose-6-phosphate dehydrogenase defficiency is a genetically determined anomaly most widely spread in man (Tzoneva 1982, WHO 1967). It plays an essential role in the pathogenesis of a great number of diseases, particularly those characterized by hemolytic crisis and anemia. An individual sensitivity of G6PD-deficiency carriers to a great number of medicaments, such as analgetics, antimalarial drugs, sulfonamides and sulfones, some antibiotics and other medicaments, as well as to foodstuffs (*Vicia faba*), is universally known (WHO 1967).

The polymorphism of enzyme deficit consists in the inheritance of a series of semidominant alleles, linked with the sex *X* chromosome.

The geneography of G6PD-deficiency corresponds to the zones of paludal wide spread (Luzzatto 1977, Motulsky 1974, Siniscalco 1966, Tzoneva 1982). It has been found that enzymopenic erythrocytes are resistant to the action of *Plasmodia* (Luzzatto 1977). In the past malaria was endemic in Bulgaria. For example, in

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1946 when antimalarial programmes were started on a wide scale, morbidity from this infection reached 20.7 per 1000 persons. Malaria was most frequent in lowland river areas, but was unknown in the areas situated over 1000 m above sea level. This suggests a wide spread of G6PD-mutant alleles in our population. Investigations on the frequency, polymorphism and clinical expression of the G6PD genes in Bulgarian population are important in working out public health programme of prophylaxis from this disease.

During the past 15 years we have performed extensive studies on the genetic status of Bulgarian population, including the problem of G6PD-deficiency. This report presents our results on the G6PD-deficiency in Bulgaria.

MATERIAL AND METHODS

The population study covered 20 809 persons, aged above 10 years and inhabiting the following 10 districts: Varna, Tolbuhin, Razgrad, Pleven, Vratza, Vidin, Sofia-city, Plovdiv, Blagoevgrad, Smoljan (Fig. 1). Comparative studies were carried out on

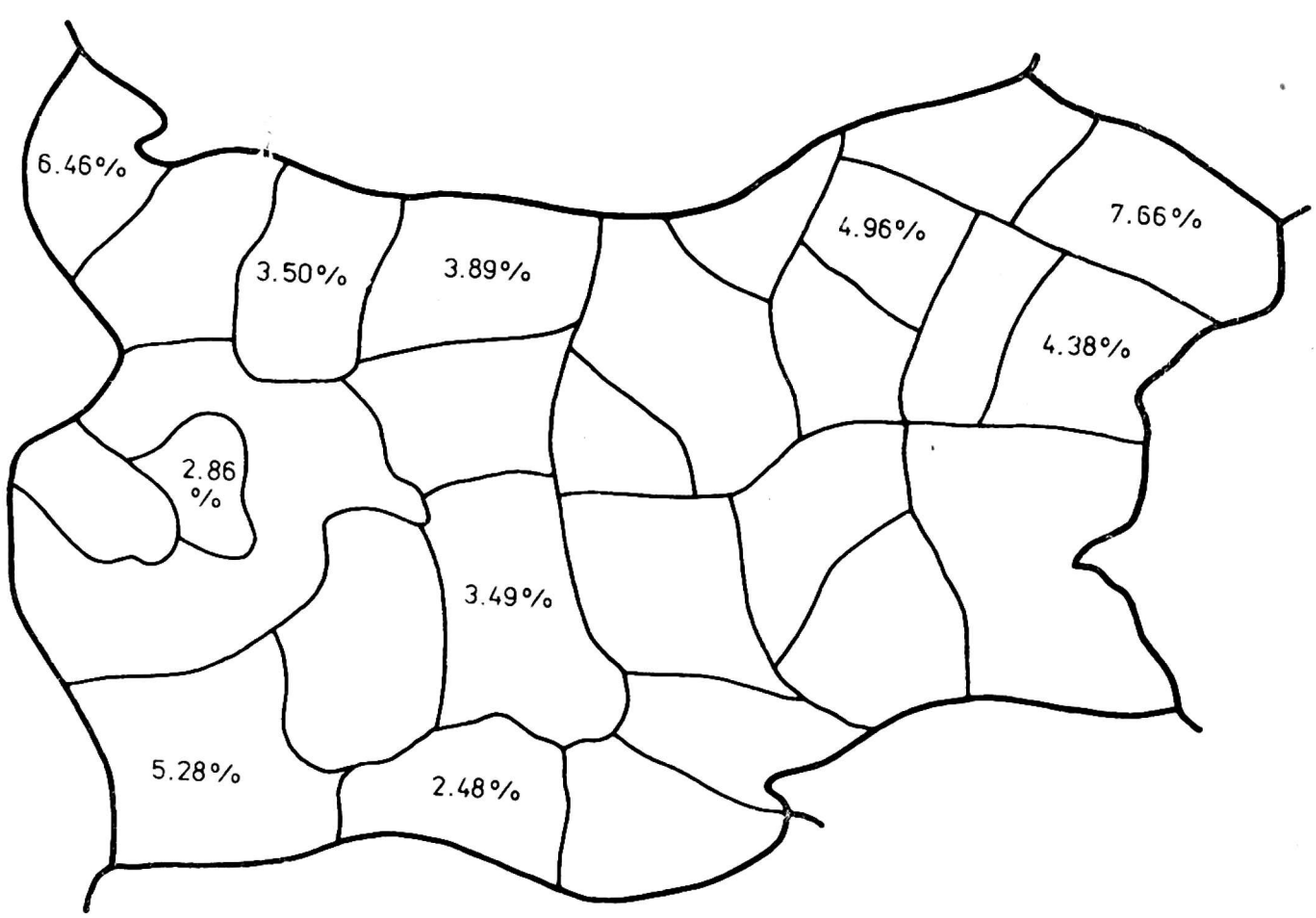

Fig. 1. Distribution of the G6PD deficiency in some areas of Bulgaria

2000 unselected newborns, and on 536 newborns with neonatal hyperbilirubinemia and total exsanquinotransfusion (selected group) (Tzoneva 1982, Tzoneva et al. 1982).

The G6PD mutant variants isolated from 117 hemizygous carriers were identified. Part of them — 38, were discovered after a mass-screening, and 79 were selected after clinical manifestation of the enzyme deficiency (favism, neonatal hyperbiliru-

binemia, drug-induced hemolytic anemias and chronic nonspherocytic hemolytic anemias) (Shatskaya et al. 1980; Toncheva et al. 1982).

Various population groups selected from the areas of different altitudes were used as a model for the analysis of malaria hypothesis. For that purpose the individuals were divided into three groups according to the altitude of towns and villages above sea level. Group I — 0 - 199 m, Group II — 200 - 999 m, Group III — 1000 m and over that height. A statistical analysis was made by the χ^2-method (Tzoneva et al. 1980).

For detection of G6PD-deficiency, including identification of heterozygotes, we applied the methods of Brewer et al. (1960), Töenz and Rossi (1963), as well as fluorescent screening (Beutler, Mitchell 1960).

The WHO programme for physico-chemical and biochemical investigation of a mutant enzyme, obtained from hemizygous Gd^- carriers, was used (WHO 1967). The programme covers the following parameters:

— starch gel electrophoresis in two buffer systems: tris-EDTA-borate (pH 8.6) and phosphate (pH 7.0);

— determination of the Michaelis constant for two substrates: glucose 6-phosphate and deamino-NADP;

— determination of the velocity of utilizing the substrate analogues: 2-desoxy--glucose-6-phosphate and deamino-NADP;

— thermolability evaluation, and

— pH-dependence determination.

As controls were used samples from erythrocytes of healthy donors with normal G6PD-activity. The substrates and substrate analogues were provided by Sigma (USA). The investigation was carried out at 25°C using VSU-2P spectrophotometer.

RESULTS

Our results are given in Tables 1, 2, 3, 4, 5, 6 and in Figures 1 and 2.

Table 1 and Fig. 1 represent the results of our population study on the G6PD-deficiency in different areas of Bulgaria. The phenotype frequency of the G6PD-defi-

Table 1. Incidence of G6PD-deficiency in some districts of Bulgaria

Districts	Subjects N	Individuals with G6PD-deficiency (%)			Phenotype frequency	Gene frequency
		Hemizygotes	Heterozygotes	Homozygotes		
Tolbuchin	1840	3.58	11.47	0.55	7.66±1.24	0.0358
Varna	1568	4.88	3.11	0	4.38±2.24	0.0488
Vratza	2600	1.70	5.65	0	3.50±0.71	0.0170
Plovdiv	3270	1.52	5.65	0	3.48±0.63	0.0152
Blagoevgrad	947	1.03	11.48	0.55	5.28±1.42	0.0103
Blagoevgrad	1377	1.06	4.32	0	2.54±0.83	0.0106
Vidin	1455	6.10	6.99	0	6.47±1.27	0.0610
Pleven	1156	1.61	5.87	0.17	3.89±1.10	0.0161
Smoljan	1770	1.18	4.12	0.13	2.48±0.73	0.0118
Razgrad	1613	6.32	2.23	0	4.96±1.06	0.0632
Sofia-City	3213	2.51	3.31	0	2.86±0.58	0.0251
Total	20809 Mean:	2.86	5.64	0.11	4.10±0.27	0.0286

ciency is 4.10±0.27% in persons, and the gene frequency is 0.0286. Significant differences were revealed in the frequency of the Gd⁻ mutation between people from different areas. The largest number of G6PD-deficient individuals was found in the low-land areas, and a lower number — in the high-land areas.

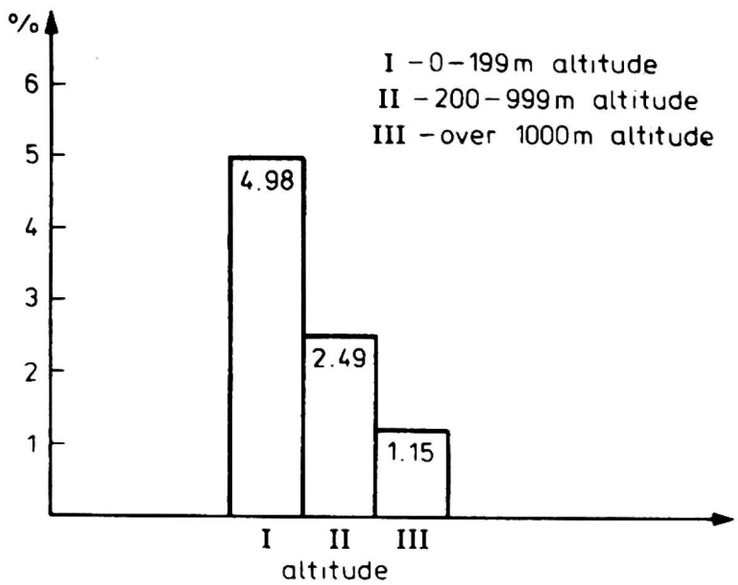

Fig. 2. The significant difference in the prevalence of the G6PD deficiency in human population living at an altitude of over 1000 m

Fig. 2 shows a significant difference in the prevalence of the G6PD-deficiency in human population living at an altitude of over 1000 m ($\chi^2=7.87$; $\chi^2=7.13$; $0.001<P<0.01$), where it was three times lower than that in people living below 1000 m. The higher number of G6PD-deficient individuals in the low-land areas (4.98% and 2.49%) and the lower number in the high-land areas (1.15%) confirm the malaria hypothesis (Tzoneva et al. 1980, Tzoneva 1982).

Table 2. Incidence of G6PD-deficiency in nonselected newborns, newborns with neonatal hyperbilirubinemia and adults

Group	Number of individuals			G6PD-Deficiency			Total No. %
	Total	Males	Females	Hemi-zygotes	Homo-zygotes	Hetero-zygotes	
Nonselected newborns	2 000	1 011	989	62	0	94	156 7.8
Selected newborns with neonatal hyperbilirubinemia	536	309	227	36	0	47	83 15.49
Adults	20 809	11 812	8997	338	9	507	854 4.10

Table 2 shows the frequency of the G6PD-deficiency in nonselected newborns and in a selected group with neonatal hyperbilirubinemia. The transmutation was two-fold higher in newborn babies with hyperbilirubinemia (7.18% and 15.49%) which points out to a possible role of the genetic defect in pathogenesis of neonatal jaundice. The incidence of the Gd⁻ mutation was higher among newborns than among adults (7.8% and 4.10%). This fact suggests the pressure of natural selection upon the carriers of genetic deffect (Tzoneva et al. 1982).

Our studies on genetic polymorphism and its clinical expression are presented in the tables 3, 4, 5 and 6. Tables 3 and 4 present genetic variants of G6PD discovered in Bulgarian population. A great number of G6PD mutant enzymes have been iden-

Table 3. G6PD-variants in Bulgarian population

G6PD variants	Number of subjects	%	Activity in RBC (%)	Electro-phoretic mobility	K_mG6P M	Deoxy--G6P (%)	GAL-6P (%)	deNADP (%)	Thermo-stability	pH optimum
Mediterranean	70	59.83	3.4-8.8	100	11.5-12.5 15.6-26.0	50±5	44±3	280-320	reduced	biphasic
Corinth	20	17.09	3-8	100	13.8-29.8	27±9	24±6	50-58	reduced	biphasic
Fayoum	5	4.25	0-12	100	42-79	55±15	42±15	184-288	reduced	biphasic
Seattle	8	6.84	10-18	90	20-25	9±3	10±2	105-143	normal	biphasic
Ohut II	2	1.71	5-22	90-95	50.3-57	73	6-7	67-80	reduced	normal
Boston	2	1.71	5.5-6	100	13-14	37-40	21-25	175-177	reduced	biphasic
Panay	1	0.85	7.4	90	23.7	11	9.7	137	reduced	sl. biph.
Kilgor	3	2.56	3-6	100	5.3-10.4	40±3	32±2	—	normal	sl. biph.
Poznan	1	0.85	3.0	90	26.0	45	—	245	reduced	sl. biph.
Tarsus	1	0.85	8.0	100	16.1 52.6-62	35	26	236	reduced	biphasic
G6PD B	30		100	100	65-70			42.3-50	normal	normal

Table 4. New G6PD-variants in Bulgarians

Variant G6PD	Number of subjects	%	Activity in RBC %	Electro-phoretic mobility	K_m G6P M	Deoxy-G6P	GAL6P	deNADP	Thermo-stability	pH optimum
Petrich	1	0.85	4.7	100	17	6.2	7.5	75	reduced	sl. biphasic
G. Delchev	1	0.85	1.8	100	16	14	20	96	increased	sl. biphasic
Rudosem	1	0.85	6.3	100	6.9	136.7	247	147	normal	biphasic
Nedelino	1	0.85	43	100	57.14	11.9	6.64	55.9	normal	biphasic

tified — 14 G6PD variants from 117 persons studied. The most common variant with a polymorphic frequency in Bulgarian population is G6PD Mediterranean type. Out of all the persons studied 59.63% are carriers of Mediterranean type, 17.09% of G6PD Corinth, 6.84% — of G6PD Seattle, 4.25% — of G6PD Fayoum, and 11.97% are sporadic variants. Four new variants have been identified — G6PD Petrich, G6PD Gotze Delchev, G6PD Rudozem and G6PD Nedelino (Shatskaya et al. 1980).

Table 5 represents genetic G6PD variants discovered in population-genetic studies on healthy persons and on a group of persons with clinical manifestations.

Table 5. G6PD variants in population and in selected group

Variants of G6PD	Total number	%	Population		Selected group	
			N	%	N	%
Mediterranean	70	59.83	14	36.84	56	70.89
Corinth	20	17.09	6	15.79	14	17.72
Fayoum	5	4.25	5	13.16	—	—
Kilgor	3	2.56	3	7.89	—	—
Ohut II	2	1.71	1	2.63	1	1.27
Boston	2	1.71	2	5.26	—	—
Panay	1	0.85	1	2.63	—	—
Tarsus	1	0.85	1	2.63	—	—
Poznan	1	0.85	1	2.63	—	—
Petrich	1	0.85	1	2.63	—	—
Gotze Delchev	1	0.85	1	2.63	—	—
Rudosem	1	0.85	1	2.63	—	—
Nedelino	1	0.85	1	2.63	—	—
Seattle	8	6.84	—	—	8	10.13
Total	117	100	38	100	79	100

A great variety of G6PD variants is observed in the general population — 13 out of 38 persons studied. Out of them 36.84% are carriers of the Mediterranean type, 15.79% — of G6PD Corinth, 13.16% G6PD Fayoum and 7.89% of G6PD Kilgore. The remaining variants occur only in sporadic cases. Asymptomatic carriers of Gd- genes were 38 out of 117 persons studied.

A selected group of 79 persons with clinical manifestations was found to have 4 variants of G6PD: G6PD Mediterranean (70.89%), G6PD Corinth (17.72%), G6PD Seattle (10.13%), and G6PD Ohut II (1.27%). As seen from these data only these four variants are of primary importance for clinical practice in our country. Out of them only two variants — Mediterranean and Corinth, are widely distributed in the population, whereas G6PD Seattle is a rare one, detected only in persons with clinical manifestations. G6PD Ohut II is a sporadic variant, too.

Table 6. Distribution of G6PD variants in Gd- persons with different clinical manifestations

Variants of G6PD	Number of subjects	Favism		Neonatal hyperbilirubinemia		Drug induced haemolytic anaemias		Chronic haemolytic anaemias		Combined clinical forms	
		N	%	N	%	N	%	N	%	N	%
Mediterranean	56	30	53.57	7	12.5	8	14.29	7	12.5	4	7.14
Corinth	14	9	64.29	1	7.14	2	14.29	—	—	2	14.29
Seattle	8	2	25.0	3	37.5	1	12.5	1	12.5	1	12.5
Ohut II	1	1	—	—	—	—	—	—	—	—	—
Total	79	42	53.16	11	13.92	11	13.92	8	10.13	7	8.86

Table 6 shows the distribution of G6PD variants in the Gd- persons with different clinical manifestations: 42 persons with favism, 11 — with neonatal hyperbilirubinemia, 11 — with drug-induced hemolytic anemias, 8 — with chronic hemolytic anemias, and 7 persons with combination of the mentioned forms.

The Mediterranean variant is the most frequent and is of the greatest clinical importance in our country. On the whole, 70.89% of the G6PD-deficient persons with clinical manifestations are carriers of this mutant alloenzyme. Its pathology is the most serious and variable: favism (53.57%) — of the carriers of Mediterranean variant in selected group, neonatal hyperbilirubinemia (12.5%), drug-induced hemolytic anemias (12.5%), as well as combined manifestations (7.14%).

G6PD Corinth is almost equally distributed in the general population (15.72%) and in the group of persons with clinical manifestations (17.72%). It occurs in patients with favism (64.29%), neonatal hyperbilirubinemia (7.14%), drug-induced hemolytic anemias (14.29%) and in combination of the mentioned forms (14.29%).

G6PD Seattle is distributed in the group of persons with favism (25%), neonatal hyperbilirubinemia (37.5%), drug-induced hemolytic anemias (12.5%), chronic hemolytic anemias (12.5%) and in combined clinical forms (12.5%).

Variant Ohut II was identified in a patient with favism, only.

G6PD Mediterranean and G6PD Corinth are associated predominantly with favism, whereas G6PD Seattle — with neonatal hyperbilirubinemia. In the group of persons with chronic hemolytic anemia two variants were detected — G6PD Mediterranean and G6PD Seattle.

These data are of a significant applicable value in our clinical practice, taking into account that the G6PD Mediterranean type constitutes 36.84% and G6PD Corinth type — 15.79% of the general population.

From the results reported here the following conclusions can be drawn:

1. Gene mutation controlling the synthesis of G6PD is widely distributed among Bulgarian population — 4.10%.

2. Difference in the mutation frequency between adults and newborns shows the action of natural selection (4.10 vs. 7.80%).

3. The G6PD-deficiency takes part in pathogenesis of neonatal jaundince in Bulgarian population. In newborns with neonatal hyperbilirubinemia 15.49% have G6PD-deficiency rather than mother child incompatibility.

4. As many as 14 G6PD mutant alleles are distributed among Bulgarian population. The most polymorphic is the Mediterranean type.

5. Mediterranean, Corinth, Seattle and Ohut II variants have clinical manifestations.

6. A significant difference in G6PD-deficiency between people living at an altitude over 1000 m and below 1000 m (three times lower) confirms the malaria hypothesis.

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PROBLEMY MEDYCZNE ZWIĄZANE Z NIEDOBOREM AKTYWNOŚCI G6PD W POPULACJI MIESZKAŃCÓW BUŁGARII

Streszczenie

Przypadki niedoboru G6PD stanowią w Bułgarii problem o dużym znaczeniu społecznym. Na podstawie badań przeprowadzonych w Katedrze Genetyki Medycznej, obejmujących 20 809 osób, stwierdzono występowanie niedoboru G6PD z częstością 4.1% (S.D.O. 27%), co odpowiada występowaniu alleli Gd^- z częstością 0.0286. W badanej grupie stwierdzono występowanie 14 odmian niedoboru G6PD, przy czym najczęściej (w ponad 50% wszystkich przypadków) występował typ śródziemnomorski. Cztery spośród stwierdzonych odmian niedoboru G6PD nie były dotychczas opisane.

W grupie noworodków wykazujących objawy przedłużonej żółtaczki niedobór G6PD stwierdzono w 17.31% przypadków.

Opisywany defekt metaboliczny zwiększa śmiertelność okołoporodową i noworodków wśród potomstwa nosicieli genu, co można uznać za objaw zmniejszonego przystosowania.

Na poziomie ekspresji klinicznej stwierdza się również liczne odmiany defektu, jak np. fawizm, niedokrwistość polekową, niedokrwistości hemolityczne, w których czynnik wywołujący nie został ustalony, niedokrwistości przewlekłe itp.

Najważniejszą przesłanką wskazującą na praktyczne znaczenie wyników przedstawionych badań jest częstość występowania niedoboru G6PD w badanej populacji.

НЕДОСТАТОК G6PD В БОЛГАРСКОЙ ПОПУЛЯЦИИ И НЕКОТОРЫЕ, СВЯЗАННЫЕ С ЭТИМ ПРОБЛЕМЫ МЕДИЦИНЫ

Резюме

Недостаток G6PD — это проблема большой важности с медицинской и социальной точек зрения для болгарской популяции. Популяционные исследования болгарских учёных, прпводившиеся на 20 800 особях, обнаружили частоту фенотипа $4.10 \pm 0.27\%$ и частоту Gd^- гена 0.0286 в Болгарии. Существует большой генетический полиморфизм Gd^- вариантов. Мы обнаружили 14 вариантов, из которых Средиземноморской был наиболле частым — более 50%. Четыре новых варианта недостатка G6PD были загеристрированы впервые.

Обнаружено, что недостаток G6PD у новорождённых с неонатальной гипербилирубинемией является этиологическим фактором в 17.31%.

Этот генетический дефект поражает репродуктивные процессы и витальность популяции, увеличивая пренатальную и неонатальную смертность среди потомства носителей недостаточности G6PD.

Мы обнаружили сильный клиническо-генетический полиморфизм недостатка G6PD, который проявлялся как фавизм, анемии, вызванные лекарствами, и острые гемолитические анемии с неизвестным ещё провоцирующим фактором, хронические анемии и т.д.

Высокая частота и специфический генетический и клинический полиморфизм недостаточности G6PD подчёркивают большое практическое значение полученных данных.