

# Urinary pepsinogen polymorphism in Jordanians classified according to blood group, cigarette smoking habits and region\*

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## ABSTRACT

The pattern of distribution of Urinary pepsinogen A (PGA) polymorphism phenotypes in 535 normal Jordanians, with respect to sex, age, ABO blood groups, cigarette smoking, and geographical distribution, was investigated. The phenotypes were determined with polyacrylamide gel electrophoresis. Seventeen discrete phenotypes, which differ with respect to the presence and relative intensity of the PGA groups (Pg5, Pg4 and Pg3), were identified. Each phenotype was consistent for each individual. A new PGA phenotype, BC > CC, was identified. The others differed in their frequencies from those of previously studied populations. No correlation between PGA phenotypes and age and sex of individuals was observed. Pg5 was more frequent in the AB blood group. Strong Pg5 and Pg4 were found more and less frequently, respectively, in cigarette smokers compared to nonsmokers. Furthermore, phenotypes with strong Pg5 were more frequently present in individuals of the eastern desert than in those of the mountainous region.

## INTRODUCTION

Pepsinogen polymorphism has been determined in several populations: American whites (Samloff and Townes, 1970; Weitkamp and Townes, 1975; Taggart *et al.*, 1979) and blacks (Samloff *et al.*, 1973; Townes and White, 1974); English (Bowen *et al.*, 1972; Oriental (Samloff *et al.*, 1973); Norwegian (Korsnes and Gedde-Dahl, 1980); and Dutch (Frants *et al.*, 1984; Bebelman *et al.*, 1989). A number of factors have been shown to influence the mode of gene expression of these phenotypes (Pals *et al.*, 1985; Parente *et al.*, 1985; Massarat *et al.*, 1986; Bianchi-Porro *et al.*, 1988; Magni

*et al.*, 1988; Malesci *et al.*, 1988; Germana *et al.*, 1990; Lanas *et al.*, 1990).

To the best of our knowledge, there is no information about pepsinogen polymorphism in any Arab population.

## MATERIAL AND METHODS

Urine samples were collected randomly from 535 apparently healthy Jordanian volunteers (276 males and 259 females) from various regions of Jordan, of different ages and ABO blood groups (they were informed about the genetic purpose of the study). The urine samples were stored at 4°C with sodium azide at 0.03% (w/v) until analyzed (within one week).

Polyacrylamide gel electrophoresis was performed under a nondenaturing discontinuous system

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according to a modification of the system described by Taggart *et al.* (1978) and Taggart and Samloff (1987). The separating gel (11 cm) consisted of 8% acrylamide, 0.25% bisacrylamide, and 35 mM Tris-HCl buffer, pH 7.5. The stacking gel (3 cm) contained 2.5% acrylamide, 0.6% bisacrylamide, 20% sucrose and 25 mM Tris-H<sub>3</sub>PO<sub>4</sub> buffer, pH 5.5. The upper electrode buffer was one-half the concentration of the lower electrode buffer (8.25 mM Tris, 30 mM diethyl barbituric acid, pH 7.5). Urine samples were diluted 5:1 with 80% sucrose solution containing 0.01% bromophenol blue as a marker. Usually a 30 to 50 µl sample of the diluted urine was subjected to electrophoretic separation on 0.75 mm-thick slab gels. Electrophoresis was performed in a Pharmacia vertical gel electrophoresis apparatus under a constant current of 10 mA/gel for 3 h at constant temperature, 20°C.

The gels were stained for acid protease activity as described by Taggart *et al.* (1978). The gels were incubated in 0.7% hemoglobin solution for 15 min., after which they were incubated in 0.1 M HCl solution at 37°C for 1 h. They were then stained in Coomassie brilliant blue solution for 1.5 h, and destained in 10% acetic acid solution. The pepsinogen phenotype nomenclature of Taggart *et al.* (1979) as modified by Frants *et al.* (1984) was used to designate the observed PGA phenotypes in this study.

**Statistics** - Correlation between PGA phenotypes and sex, age, ABO blood groups, cigarette smoking, and geographical distribution was tested using the chi-square test of independence. The data were analyzed with the computer program SAS. The association was considered significant when P < 0.05.

## RESULTS

There was considerable variation in PGA isozyme patterns. The individuals could be categorized into 17 different phenotypes (Figure 1) based on the presence or absence and relative intensity of the main PGA isozymogens, Pg3, Pg4 and Pg5. PGA phenotypes remained constant for each individual during a 24 h

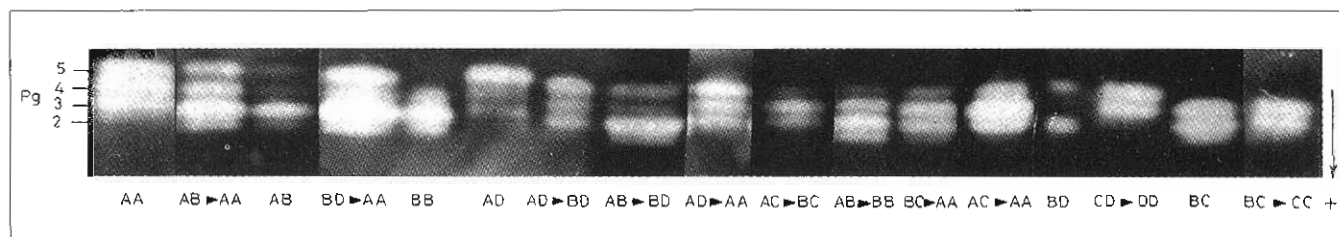
period and during a one year period. The highest PGA phenotype frequency was observed in individuals carrying the AB > BD phenotype; while phenotypes BD, BC > CC, AC > BC, CD > DD had the lowest frequency (< 1%) (Figure 2). Phenotypes with intense Pg3 activity were most frequently present, while phenotypes with intense Pg4 were least frequent; Pg5 was more frequently absent when compared with Pg4 and Pg3 (Table I).

For statistical analysis, PGA phenotypes were classified into groups based on the degree of intensity of the main PGA isozymes, Pg3-5. The distribution of PGA phenotypes showed no significant differences with respect to sex (Table II) or age (Table III). However,

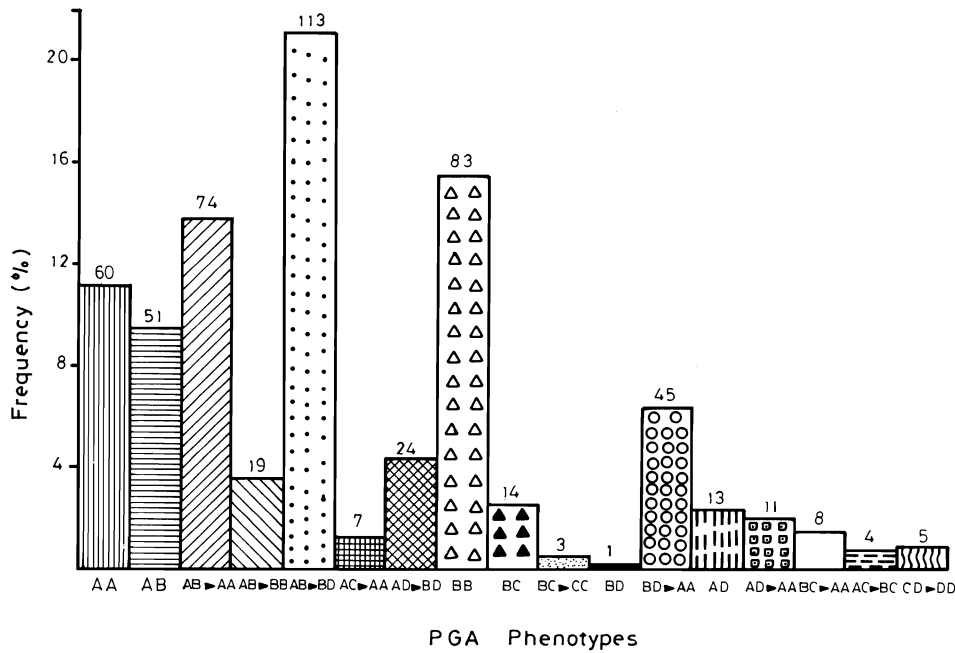
**Table I** - Comparison of PGA phenotype groups classified on the basis of the main PGA isozymogens: Pg5, Pg4 and Pg3, and their frequencies in this study with those of other studies.

PGA phenotype group	Present study %	Other studies			
		F-1984 %	B-1989 %	T-1979 %	K-1980 %
a. Pg5 <sup>+</sup>	39.6	31.0	13.7	5.6	31.2
Pg5	41.7	57.1	73.5	76.7	56.4
Pg5 <sup>-</sup>	18.7	11.9	12.8	17.7	12.3
b. Pg4 <sup>+</sup>	7.7	84.2	28.2	12.1	13.2
Pg4	92.2	15.5	70.1	72.1	84.1
Pg4 <sup>-</sup>	0.2	0.3	1.7	15.8	2.7
c. Pg3 <sup>+</sup>	82.1	65.5	60.7	60.9	94.3
Pg3	17.0	26.2	29.1	38.1	4.8
Pg3 <sup>-</sup>	0.9	8.3	10.3	0.9	1.0
N	535	336	117	215	948

<sup>+</sup>Present as intense band.  
Pg5, Pg4, Pg3 present as intermediate or weak bands.  
<sup>-</sup>Absent band.  
F-1984: Frants *et al.* (1984) (Dutch population).  
B-1989: Bebelman *et al.* (1989) (Dutch population).  
T-1979: Taggart *et al.* (1979) (American White population).  
K-1980: Korsnes and Gedde-Dahl Jr. (1980) (Norwegian population).



**Figure 1** - Urinary pepsinogen A phenotypes in Jordanians.



**Figure 2** - Distribution of pepsinogen A phenotypes in Jordanians. The number in each column indicates the number of individuals having that particular phenotype.

**Table II** - Frequencies of pepsinogen A phenotype groups according to sex. PGA phenotypes were grouped based on the relative intensity of Pg5 band.

PGA phenotype group	Males	Females
	N = 259	N = 276
	%	%
Pg5 <sup>+</sup>	39.8	39.4
Pg5	40.9	42.4
Pg5 <sup>-</sup>	19.3	18.1

The overall  $\chi^2 = 0.17$ ; P = 0.92.

**Table III** - Frequencies of pepsinogen A phenotype groups according to age. PGA phenotypes were grouped based on the relative intensity of the Pg5 band.

PGA phenotype group	Age (years)		
	< 25	26 - 50	> 50
	N = 363	N = 140	N = 32
	%	%	%
Pg5 <sup>+</sup>	38.8	39.3	50.0
Pg5	43.0	41.4	28.1
Pg5 <sup>-</sup>	18.2	19.3	21.9

The overall  $\chi^2 = 2.74$ ; P = 0.60.

all individuals with AB blood type had Pg5 (Table IV). No correlation was observed among the distributions of PGA phenotype groups when the phenotypes were classified based on the degree of intensity of Pg4 and Pg3 bands and their blood groups. The distribution of Pg5 phenotype groups were significantly different in the two regions of Jordan (Table V). This difference includes the more frequent presence of phenotypes with intense Pg5 (Pg5<sup>+</sup>) and less frequent intermediate or weak Pg5 (Pg5) in the eastern desert. Phenotypes without Pg5 (Pg5<sup>-</sup>) were almost equally distributed in these regions.

**Table IV** - Frequencies of PGA phenotype groups based on ABO blood groups. PGA phenotypes were grouped based on the relative intensity of the Pg5 band.

PGA phenotype group	Blood group			
	A	B	AB	O
	N = 84	N = 33	N = 26	N = 96
	%	%	%	%
Pg5 <sup>+</sup>	40.5	36.4	34.6	35.4
Pg5	36.9	45.5	65.4	41.7
Pg5 <sup>-</sup>	22.6	18.2	0	22.9

The overall  $\chi^2 = 10.25$ ; P = 0.12.

**Table V** - Frequencies of PGA phenotype groups in two different regions of Jordan. PGA phenotypes were grouped on the basis of the relative intensity of the Pg5 band.

PGA phenotype group	Mountainous region N = 485	Eastern desert region N = 50
I. Pg5 <sup>+</sup>	38.1	54.0
II. Pg5	43.5	24.0
III. Pg5 <sup>-</sup>	18.4	22.0

The overall  $\chi^2 = 7.34$ ;  $P = 0.03$ .

No significant differences were found in the geographical distribution of Pg4 and Pg3 phenotype groups.

The distribution of PGA phenotype groups in 67 cigarette smokers indicated that phenotypes with an intense Pg5 band were present more frequently and phenotypes with intermediate or weak Pg5 were less frequently present in smokers than in nonsmokers ( $P = 0.02$ ) (Table VI). In addition, phenotypes with a Pg4 band were present less frequently in smokers than in nonsmokers ( $P = 0.04$ ) (Table VI). The distribution of Pg3 phenotype was not significantly affected in these individuals compared with nonsmokers. Furthermore, only the frequency of AB phenotype was greater in nonsmokers (10.5%) than in smokers (3.0%) ( $P < 0.05$ ) (Figure 2).

**Table VI** - Effect of cigarette smoking on PGA phenotype groups. PGA phenotypes were grouped based on relative intensity of bands.

PGA phenotype group	Nonsmokers N = 468 %	Smokers N = 67 %
a - I. Pg5 <sup>+</sup>	37.8	52.2
II. Pg5	43.4	29.9
III. Pg5 <sup>-</sup>	18.8	17.9
b - I. Pg4 <sup>+</sup>	8.6	1.5
II. Pg4	91.2	98.5
III. Pg4 <sup>-</sup>	0.2	0

a. The overall  $\chi^2 = 5.67$ ;  $P = 0.06$ . For I v.s. II,  $\chi^2 = 5.60$ ;  $P = 0.02$ .

b. The overall  $\chi^2$  is not valid. For I v.s. II,  $\chi^2 = 4.14$ ;  $P = 0.04$ .

## DISCUSSION

Only PGA isozymogens Pg2, Pg3, Pg4, and Pg5 were observed. Pg1 was absent since it is a minor component of gastric mucosa (< 1% of the total PGA)

(Taggart and Samloff, 1987). Pg5S isoenzyme (Pg5 slow) which was found cathodally to Pg5 and belongs to the pepsinogen A group (immunochemically positive to PGA) and was observed in other populations (Taggart *et al.*, 1979; Kornes and Gedde-Dahl, 1980; Frants *et al.*, 1984) was absent in our sample. PGC was absent in the urine of all individuals examined, possibly due to the presence of high tubular reabsorption (Ten Kate *et al.*, 1988; 1989a,b,c). This could be due to minor molecular structural differences in both pepsinogens. It has been suggested that the Pg2 fraction is a posttranslational modification of Pg3 fraction, which is a product of the primary B gene (Kornes and Gedde-Dahl, 1980; Frants *et al.*, 1984; Defize *et al.*, 1985). This report confirms and is in agreement with their finding as the intensity of the Pg2 band was dependent on the degree of intensity of Pg3 band, and the Pg2 band was not observed when the Pg3 band was absent. Also it increased in stored urine samples. For this reason, fraction 2 has not been included in phenotyping studies. Evers *et al.* (1989) and Bebelman *et al.* (1989), in their studies using PGA cDNA probes and human genomic DNA - Hind III or Ava II and EcoR I restriction fragment length polymorphisms (RFLPs), have confirmed that no gene codes for the Pg2 fraction.

Our study showed 17 discrete phenotypes in the Jordanian population. These phenotypes are characterized by the presence of the BC > CC phenotype, which was not observed in any other studies. This phenotype is actually the same as the BC phenotype, with A relatively high intensity of the Pg4 band, compared with the Pg3 band. However, the frequency of this phenotype is low (0.6%). Several phenotypes reported by other investigators were not found in our study (*i.e.* CC, AD > CD, AC, CD and CD > AA phenotypes).

The AB > BD phenotype had the highest frequency in Jordanians, while it has been reported to be very low in other populations (Table I). The frequency of the AB phenotype was lower in this study compared with other studies (Table I). Also the frequency of AB > AA phenotype was higher in this study than reported in other populations. This discrepancy may be due to the difficulty in classification of the electrophoretic pattern as phenotype AB or AB > AA, since these phenotypes were very similar. Phenotypes such as BD, AC > BC and CD > DD had the lowest frequencies (< 1%) and were similar to those in other studies.

In the report of Pals *et al.* (1985), phenotypes with Pg5 were more frequent in males than in females; we have not observed this phenomenon in our study. However, both reports are in concordance with respect

to the distribution of PGA phenotypes according to age *i.e.* no difference. Furthermore, Pals *et al.* (1985), in their study, found no difference in the distribution of PGA phenotypes with respect to ABO blood type. In our study, we have found that phenotypes with the Pg5 isozymogen were significantly more frequently present in individuals with AB blood group than other blood groups; and no individual with this blood phenotype lacked this Pg5 isozymogen.

The distribution of PGA phenotypes according to geographical residence indicates that there may be a gene segregation in Jordan population. For instance, the people of the eastern region (Bedouins) have more frequent intense Pg5 and less frequent intermediate or weak Pg5 isozymogen than those who live in the mountainous region. No such geographical distribution study has been reported in the literature. An environmental factor could not be ruled out as a reason for this difference. It is interesting to note that in this region there are less cigarette smokers than in other regions of the country.

Cigarette smoking increases pepsinogen levels (Massarat *et al.*, 1986; Magni *et al.*, 1988; Germana *et al.*, 1990; Lanas *et al.*, 1990), and its effect on peptic ulcer has been demonstrated (Malesci *et al.*, 1988). However, the effect of cigarette smoking on distribution of PGA phenotypes was not studied before. We found that phenotypes with intense Pg5 isozymogen were more frequent and phenotypes with intense Pg4 isozymogen less frequent in cigarette smokers than in nonsmokers. The effect of the period of smoking is not known. Cigarette smoking may affect PGA expression, leading to more synthesis of Pg5 and less synthesis of Pg4. The previously observed high level of serum PGA in cigarette smokers is possibly due to an increase in Pg5 isozyme expression (Parente *et al.*, 1985; Massarat *et al.*, 1986; Magni *et al.*, 1988; Bianchi-Porro *et al.*, 1988).

## RESUMO

Investigou-se polimorfismo pepsinogênico A urinário em 535 Jordanianos normais com respeito a sexo, idade, grupo sanguíneo ABO, hábito de fumar e distribuição geográfica. Os fenótipos foram determinados em eletroforese gel poliacrilamido. Foram identificados 17 fenótipos discretos, que diferem no que diz respeito à presença e intensidade relativa do grupo PGA (Pg5, Pg4 e Pg3). Cada fenótipo foi consistente e caracterizado para cada indivíduo. Destes foi identificado um novo fenótipo PGA, BC > CC; outros foram similares, mas diferiram em suas freqüências daquelas populações previamente estudadas. Não foi observada correlação entre fenótipos PGA, idade e sexo dos indivíduos. A Pg5 foi mais

freqüente no grupo sanguíneo AB. Encontrou-se forte Pg5 e Pg4 mais ou menos freqüente, respectivamente, mais em fumantes do que não fumantes. Além disso, fenótipos com forte Pg5 foram mais freqüentes em indivíduos do deserto leste do que nos da região montanhosa.

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