G6PD DEFICIENCY AND FAVA BEAN CONSUMPTION DO NOT PRODUCE HEMOLYSIS IN THAILAND

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Abstract. Favism, a hemolytic condition associated with fava bean consumption among the glucose-6-phosphate dehydrogenase (G6PD) deficient persons, is well described in the Middle East and Mediterranean areas. However, it is not well documented among the Thais or other Southeast Asians. It is possible that it does exist but that hemolysis which develops is of very minor degree and thus escapes clinical detection. This cross-sectional study hypothesizes that if the fava bean and G6PD deficiency interact in the Thai population, they should cause a significant difference in hematocrit level. The study was carried out in a community hospital in a malaria endemic area. We found that there was a trivial difference of the hematocrit (~1%) which was too small to warrant any clinical significance after controlling for the extraneous effects of age, sex, use of malaria chemoprophylaxis, falciparum infection, use of analgesics/antipyretics and admission status of the patients ($p = 0.668$). This may be due to the presence of different G6PD mutants to those found elsewhere or due to different consumption patterns of fava beans among the Thais compared to people in other areas with high prevalence of G6PD deficiency.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is known to be one of the most common genetic abnormalities involving erythrocytes in man. This enzyme deficiency has over 150 allelic variants, which are classified according to their electrophoretic characteristics, enzymatic activity or clinical severity (Beutler, 1978). The gene for G6PD is X-linked (Childs and Zinkham, 1959). There are many circumstances that can shorten the life-span of the G6PD deficient erythrocytes, eg certain drugs, food such as the fava bean (*Vicia faba L.*, Kay, 1979; Schneider, 1989), neonatal period and certain conditions of stress, such as infection. Drug-induced hemolysis is accompanied by the formation of Heinz bodies, particles of denatured hemoglobin and stromal protein. Cells containing Heinz bodies have difficulty in traversing through the splenic pulp and are rapidly eliminated from the circulation. Other mechanisms of hemolysis are less well understood (Williams, 1983). One of the classical issues about G6PD deficiency is that it is recognized as the cause of *favism* (Fraser and Nora, 1986). While the G6PD deficiency-fava bean interaction has been depicted in the Mediterranean and the Middle East regions and the mechanisms of this favism have been postulated (Hedayat *et al,* 1981; Turrini *et al,* 1985; Anderson *et al,* 1987), to the best of our knowledge, there is no well documented report on this issue in Thailand. We therefore carried out this study to assess whether such an interaction exists. Since favism is not well recognized clinically in Thailand, it may exist but the hemolysis may be too mild so that it escapes clinical detection. Our hypothesis was that if such an interaction does exist, then it should be associated with a clinically significant difference in hematocrit.

MATERIALS AND METHODS

Study site and study population

The study-design was cross-sectional (Kelsey *et al,* 1986; Rothman, 1986). Patients (n = 395) aged 16-60 years old, who attended Pong Nam Ron Community Hospital in Chanthaburi, eastern Thailand during May 1989-January 1990, were enrolled as study subjects.

Ascertainment of exposure

A structured interview questionnaire was used to assess exposures; this included age, sex, history of fava bean consumption in the previous month, history of current malaria and time since its last attack, history of malaria chemoprophylaxis and use of analgesics/antipyretics. Laboratory investigations included routine complete blood counts (CBC), ascertainment of G6PD deficiency (fluorescent spot test, Beutler, 1966); hemoglobin types (quantitative cellulose acetate electrophoresis, Weatherall, 1983); ABO-blood groups. Laboratory technicians and interviewers were masked against the study hypothesis to minimize bias.

Sample size

Considering α-error = 0.05 and statistical power (1-β) = 0.90, to detect a significant difference (2-sided) in the hematocrit of 6% assuming common population variance (σ^2) = 0.25, a minimally required sample size will be 30 in each G6PD deficient and non-G6PD deficient group respectively (Meinert and Tonascia, 1986). Therefore, the sample size of 395 in the study should be sufficient to detect such a difference if it exists and would yield a large enough number of observations for subsequent multivariable adjustments.

Data analyses

Crude association between hematocrit levels and other independent variables were assessed by *t* or *F* test where appropriate (Armitage and Berry, 1987). Multiple linear regression is used to simultaneously control for the effects of potential confounding variables and to assess for any evidence of interaction through the use of cross-product term. The regression takes the form:-

$$\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_2 + \ldots\ldots + \hat{\beta}_n x_n + \hat{\gamma}_p x_1 x_2$$

where $\hat{y}$ = hematocrit level
$\hat{\beta}_0$ = intercept term
x_1 = G6PD deficiency (1 = yes, 0 = no)
x_2 = fava bean consumption (1 = yes, 0 = no)
$x_{3...n}$ = potential confounding variables to be controlled.

Therefore $\hat{\beta}_1$ = difference in hematocrit means between the G6PD deficient group compared to the non-G6PD deficient, controlling for the effects of other co-variables in the model. The cross-product term ($\hat{\gamma} x_1 x_2$) allows the assessment of the interaction between G6PD deficiency and fava bean consumption based on hematocrit levels. After the final fit of the model was obtained, the quantile-quantile plot of standardized residuals was used to assess the goodness of fit of the model (Kleinbaum and Kupper, 1978). Epi Info version 5 (Dean *et al,* 1990) and BMDP (Dixon, 1985) statistical packages were used for analyses where appropriate.

RESULTS

Table 1 shows the comparisons of hematocrit levels across different categories of independent variables ignoring the effects of extraneous variables. Only sex and the status of admission of the patients showed statistically significant differences ($p < 0.01$). They were, however, not clinically significant in that the hematocrit levels were within normal limits.

The proportion of G6PD deficient individuals in this data set was 45/395 (11.4%) with 95% confidence limits (95% CL) = 8.3, 14.5%. In this study, the proportions of G6PD deficiency among males and females were 14.8% (95% CL = 10.5, 19.1%) and 4.5% (95% CL = 1.0, 8.1%) respectively. It can also be observed that history of fava bean consumption was not uncommon, ie, 13.2% for those who consumed 1-2 times/week compared to 3.8% for who consumed > 2 times/week. Both the G6PD deficient and the non-deficient were comparable with respect to age means ($\bar{x}$), ie, $\bar{x} \pm SD$ = 26.91 ± 9.43 years (n = 45) and 29.63 ± 10.88 years (n = 350) respectively (p = 0.107). Age did not correlate well with hematocrit in this study (r = -0.083, df = 393, p = 0.100). Crude analysis revealed that G6PD deficiency alone did not show any meaningful difference in hematocrit levels with $\bar{x} \pm SD$ = 39.0 ± 7.5% (n = 350) and 40.1 ± 8.0% (n = 45) in the non-G6PD deficient and the G6PD deficient groups respectively (p = 0.644, Table 1).

Multiple linear regression was used to assess the relationship between G6PD deficiency and hematocrit considering the effect of history of fava bean consumption in the previous month. It is also used to assess such associations while controlling for the effects of other possible confounders. Several models were assessed. The most relevant one included the effects of G6PD deficiency, history of fava bean consumption while simultaneously

Table 1

Comparisons of hematocrit levels with respect to independent variables.

Variables	n	$\bar{x}$	SD	p-value
G6PD deficiency				
Normal	350	39.0	7.5	0.644
Deficient	45	40.1	8.0	
History of fava bean consumption in the previous month				
Never	328	39.1	7.4	0.621
1-2/week	52	40.1	8.2	
> 2/week	15	37.1	7.5	
Sex				
Male	263	40.1	7.7	< 0.001
Female	132	37.0	6.7	
Time since last malaria attack				
Never	114	38.4	7.3	0.158
> 4 months	244	39.7	7.7	
2-4 months	37	37.8	6.9	
History of chemoprophylaxis				
Never	268	39.3	7.5	0.570
Ever	127	38.7	7.5	
History of analgesic use				
Never	69	38.8	7.8	0.612
Ever	326	39.0	7.5	
Falciparum infection and parasite densities				
Negative	198	39.6	8.0	0.503
1-10/10 oil fields	147	38.9	7.1	
11-100/10 oil fields	45	37.9	6.5	
1-10/oil fields	5	37.8	9.9	
Hemoglobin types				
A & others	242	39.1	7.8	0.990
EA	130	39.0	7.0	
EE	23	39.2	7.7	
ABO blood groups				
A	74	38.6	6.9	0.651
B	150	38.7	7.5	
AB	38	40.0	6.9	
O	133	39.6	8.0	
Admission status of patients				
No	204	40.1	7.2	0.009
Admitted	191	38.1	7.7	

controlling for age, sex, history of *P. falciparum* malaria, use of analgesics/antipyretics, use of malaria chemoprophylaxis and the admission status of the patients. The model with the interaction term ($\hat{Y}x_1x_2$) between G6PD deficiency and fava bean consumption was also considered (Tables 2 and 3). However, it did not provide any evidence of significant interaction ($p = 0.668$). Comparisons of $\hat{\beta}$-estimates obtained between the models *with* and *without* interaction term between G6PD deficiency and fava bean consumption are shown in Table 2. Both models are summarized in Table 3.

The model *with* interaction between G6PD deficiency and fava bean consumption showed

Table 2

Multiple linear regression estimates of the effects of G6PD deficiency and fava bean consumption on the hematocrit levels controlling for the effects of age, sex, *P. falciparum* infection, use of analgesics/antipyretics, use of malaria chemoprophylaxis and admission status. Comparisons are made between the estimates obtained with and without the G6PD deficiency and fava bean interaction term.

1. **Model with G6PD deficiency and fava bean consumption interaction**

		G6PD deficiency	
		No	Yes
Fava bean	No	-[a]	+0.5[b] (-2.0, 3.0)[c]
	Yes	-0.1 (-2.2, 2.0)	+1.9 (-16.6, 20.4)

2. **Model without G6PD deficiency and fava bean consumption interaction**

		G6PD deficiency	
		No	Yes
Fava bean	No	-	+0.7 (-1.6, 3.0)
	Yes	+0.03 (-2.0, 2.0)	+0.7 (-4.2, 5.6)

[a] Reference category
[b] indicates changes of hematocrit from reference category.
[c] 95% CL

that neither fava bean nor G6PD deficiency alone provided significant difference of hematocrit from the reference category (non-G6PD deficient and non-fava bean consumer). It can be seen that all the 95% CLs cover the null value (0). Moreover, when the 2 effects were presented together there was a slight rise of hematocrit (1.9%) but the 95% CL seemed unrealistic and is too wide to provide a reasonable estimate (-16.6, 20.4). These findings indicate that the interaction might be unrealistic and that it would be very hard to establish biological plausibility. The model *without* interaction term, however, does provide more reasonable estimates of the effects. Slight increases in the hematocrit levels across different categories of G6PD deficiency and fava bean consumption combinations may be attributed to chance ($p > 0.05$). Combined effect of G6PD deficiency and fava bean consumption does not provide any evidence of G6PD deficiency and fava bean consumption interaction (difference in hematocrit = 0.7% with 95% CL = -4.2, 5.6). Another piece of evidence that the model with interaction may be poorer than that without interaction is that the adjusted R^2 was 4.9% in the former while it was 5.1% in the latter (Table 3). This means that adding more parameters (here the interaction term) gives a worse fit. One should observe that the poor R^2 in either model indicates that there are still many variations in the hematocrit levels (> 90%) which are left unexplained by the model. Therefore, we conclude from our data that we do not have any evidence that G6PD deficiency and fava bean consumption together would produce a significant difference in the hematocrit to denote hemolysis.

DISCUSSION

Our data did not show any evidence of hemolysis resulting from G6PD deficiency and fava bean interaction. Some biases need to be ruled out before making any conclusion. Even though our study was carried out at the near-distal end of the health care system in Thailand, some selective factors may distort the association. The G6PD deficiency prevalence in this data set was similar to several others in Thailand (Kruatrachue *et al,* 1962; Flatz and Sringam 1963). This may indirectly reflect the representativeness of the study population. Ascertainment of exposure to fava beans was unlikely to be biased since the interviewers and the technicians were masked against the study hypothesis. Proper adjustment of potential confounders was also covered in the analysis. One drawback is that the data gave relatively poor

Table 3a

Two regression models used to obtain estimates in Table 2.

Model with interaction term[a]

Hematocrit = 42.5 - 0.0542 Age - 3.22 Sex - 0.97 G6PD - 1.57 Fava bean - 0.341 Falciparum - 0.885 Analgesic - 1.34 Mal. pill - 1.82 Admit + 1.47 G6PD × Fava

Variables	$\hat{\beta}$	S.E. ($\hat{\beta}$)	p-VALUE[b]
Intercept	42.528	1.833	< 0.001
Age	-0.054	0.035	0.124
Sex	-3.216	0.808	< 0.001
G6PD	-0.967	4.061	0.810
Fava bean	-1.566	3.855	0.682
Falciparum	-0.341	0.791	0.667
Analgesic	-0.885	0.985	0.369
Mal. pill	-1.342	0.817	0.102
Admit	-1.822	0.784	0.020
G6PD × Fava	1.466	3.413	0.668

[a]Overall $F_{9,385} = 3.237$, $P < 0.001$, adjusted $R^2 = 4.9\%$
[b]2-sided

Table 3b

Model without interaction term[c]

Hematocrit = 42.4 - 0.0533 Age - 3.22 Sex + 0.70 G6PD + 0.034 Fava bean - 0.379 Falciparum - 0.862 Analgesic - 1.33 Mal. Pill - 1.80 Admit

Variables	$\hat{\beta}$	S.E. ($\hat{\beta}$)	p-value
Intercept	42.439	1.819	< 0.001
Age	-0.053	0.035	0.129
Sex	-3.221	0.807	< 0.001
G6PD	0.701	1.186	0.556
Fava bean	0.034	0.997	0.976
Falciparum	-0.379	0.785	0.631
Analgesic	-0.862	0.982	0.379
Mal. pill	-1.330	0.816	0.104
Admit	-1.800	0.781	0.022

[c]Overall $F_{8,386} = 3.627$, $P < 0.001$, adjusted $R^2 = 5.1\%$
Data codings: age (years), sex (0 = male, 1 = female), G6PD deficiency (0 = normal, 1 = deficiency), fava bean (0 = no, 1 = yes), falciparum (0 = no, 1 = yes), analgesics (0 = no, 1 = yes), malaria pills (0 = no, 1 = yes), admit (0 = no, 1 = yes).

R^2 statistics, which could indicate that some unforeseen confounding variables may not have been encompassed.

The study thus confirms many anecdotal clinical observations in Thailand about the non-existence of favism in Thailand. There are two issues that may explain this observation result, first the fava bean itself and second the G6PD deficiency. Fava bean is known among the Thais as *thua-pak-ah* (Arwooth Na Lampang, personal communication). It is usually eaten as a snack not as a major food or meal as in the Mediterranean or the Middle East regions. The beans are usually roasted and sprinkled with salt, after which they are eaten in the same manner as Westerners eat peanuts. It may be possible that such mode of cooking may destroy some active substances. This statement still needs to be proved, since the G6PD deficient erythrocytes are damaged from divicine and isouramil from fava beans, due to marked alterations of the proteolytic enzyme systems in intact erythrocytes (de Flora *et al,* 1985; Morelli *et al,* 1987). It was also shown that different varie-

ties of fava bean yielded different levels of blood glutathione (Vural and Sardas, 1984). Therefore, another possibility is that fava beans in Thailand may differ from those in the Mediterranean or the Middle East. So far, since fava beans are not a popular food in Thailand, documentation on this issue is not available to us.

On the other hand, if those substances (divicine and isouramil) remain active, the G6PD deficiency among the Thais may by itself be different from those in the Mediterranean and the Middle East where favism is prevalent. The most common G6PD mutant in Thailand (~ 54%) is the Mahidol variant, whose properties are found to be very similar to those of normal G6PD; the rest are other relatively uncommon variants (Panich *et al,* 1972; Panich and Na-Nakorn, 1980). This is most likely to be an explanation in Thailand and in our study, as it is consistent with a report that different types of G6PD deficiency associate differently with favism (Calabro *et al,* 1989). The discrepancy between the Thai and the Mediterranean or the Middle East study results thus requires clarification of the different varieties of fava beans and enzymatic activities of G6PD deficiency.

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