

Acetylator phenotype in Iraqi patients with systemic lupus erythematosus

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النمط الظاهري للمؤسِّتلات لدى مرضى عراقيين مصابين بالذئبة الحمامية المجموعية

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الخلاصة: أُجريت هذه الدراسة لتحديد حالة المؤسِّتلات لدى المرضى المصابين بالذئبة الحمامية المجموعية، ومقارنة ذلك مع مجموعة مقابلة من المتطوعين الأصحاء. وتم تحديد شدة المرض باستخدام المعايير التصنيفية المعدلة للكلية الأمريكية لطب الروماتيزم، وفهرس نشاط الذئبة الحمامية المجموعية. وبعد صيام المرضى طوال الليل، تلقى كل مشارك في البحث جرعة أحادية عن طريق الفم، مقدارها 100 مغ من الدابسون. وبعد مرور ثلاث ساعات، تم تحديد نسبة الدابسون إلى أحادي أسيتيل الدابسون في بلازما الدم. وكانت نسبة تَوَأْتُر المؤسِّتلات البطيئة في المجموعات الشاهدة 73.3% ونسبة تَوَأْتُر المؤسِّتلات السريعة 26.7%. أما في مرضى الذئبة الحمامية المجموعية، فكانت نسبة تَوَأْتُر المؤسِّتلات البطيئة لديهم 78.0% والسريعة 12.0%، ولم تكن هناك مؤسِّتلات لدى 8% منهم (عدم أحادي أسيتيل الدابسون في البلازما). ولم يكن هناك أيُّ تَرَأُّط بين حالة المؤسِّتلات وشدة الإصابة بالذئبة.

ABSTRACT The study was designed to determine the acetylator status in patients with systemic lupus erythematosus (SLE) and compare it to a matched group of healthy volunteers. Disease severity was determined using the revised American College of Rheumatology criteria for classification and the SLE disease activity index. After an overnight fast, each participant received a single oral dose of 100 mg dapsone. After 3 hours, plasma dapsone/monoacetyldapsone ratio was determined. In the control group, frequency of slow acetylators was 73.3%; frequency of rapid acetylators was 26.7%. In SLE patients, frequency of slow acetylators was 78.0%; frequency of rapid acetylators was 12.0%. However, 8.0% were non-acetylators (monoacetyldapsone not detected in plasma). There was no association between acetylator status and severity of SLE.

Phénotype d'acétylation chez des patients iraqiens atteints de lupus érythémateux systémique

RÉSUMÉ Cette étude visait à déterminer le statut acétyleur chez des patients atteints de lupus érythémateux systémique et à le comparer avec un groupe apparié de volontaires sains. La gravité de la maladie a été établie au moyen des critères révisés de l'American College of Rheumatology pour la classification et le signe d'évolutivité du lupus érythémateux systémique. Après un jeûne d'une nuit, chaque participant s'est vu administrer une dose unique de 100 mg de dapsone par voie orale. Après 3 heures, on a déterminé le rapport plasmatique dapsone/monoacétyldapsone. Dans le groupe témoin, la fréquence des acétyleurs lents était de 73,3 % ; celle des acétyleurs rapides était de 26,7 %. Chez les patients atteints de lupus érythémateux systémique, la fréquence des acétyleurs lents était de 78,0 % ; celle des acétyleurs rapides était de 12,0 %. Toutefois, 8,0 % n'étaient pas acétyleurs (monoacétyldapsone non détectée dans le plasma). Il n'y avait pas d'association entre le statut acétyleur et la gravité du lupus érythémateux systémique.

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Introduction

Acetylation is considered a major metabolic pathway in the biotransformation of a number of drugs such as procainamide, hydralazine, sulphonamides, isoniazide and dapsone and a caffeine metabolite [1]. Acetylation exhibits a genetically controlled, bimodal distribution within any given population. Individuals can be phenotyped as either slow or rapid acetylators using a test drug. Slow acetylation is inherited in an autosomal recessive fashion [1].

Polymorphic N-acetylation has been linked to variation in drug response, susceptibility to adverse reactions and increased incidence of certain spontaneous disorders, including cancer [1].

In this study, we examined the acetylator status in Iraqi SLE patients. The Iraqi population, as well as other Middle Eastern populations, is characterized by a predominance of slow acetylators [2]. It is, therefore, interesting to examine this problem in a predominantly slow acetylator population. The possible association of the acetylator state on the severity of the disease was also examined

Methods

Fifty SLE patients and 30 healthy volunteers participated in the study. Approval to conduct this study was granted by the ethical committee of the College of Medicine in the University of Baghdad. The nature of the trial was explained to the each participant and the consent of each was obtained. Excluded from this study were individuals with glucose-6-phosphate dehydrogenase deficiency or allergy to sulfonamides. These were not encountered in our sample so there were no individuals excluded from the study. No attempt was made to exclude subjects with abnormal hepatic

or renal function. None of the participants had a history of drug-induced lupus prior to phenotype determination. None were taking drugs that would interfere with acetylation nor were any on any drugs known to be polymorphically N-acetylated.

We studied all 50 consecutive, unrelated, spontaneous SLE patients attending the Department of Rheumatology in Baghdad Teaching Hospital during the study period, October 2001 to July 2002. The hospital is a tertiary care referral centre drawing patients from all over the country. The nature of the research was explained to each patient and informed consent was obtained. There were no refusals to participate in the study.

The patients were diagnosed and evaluated by a consultant rheumatologist. All SLE patients fulfilled at least 4 of the 11 revised (1982) criteria for classification of SLE of the American College of Rheumatology [3]. A thorough detailed clinical history was taken from all patients, including clinical and laboratory data.

Patients were maintained on the following treatment: 31 patients received corticosteroid therapy (prednisolone), 3 patients were on hydroxychloroquine and 4 were on cytotoxic drugs (methotaxate and cyclophosphamide); 18 patients were on no treatment. Six patients were taking more than 1 drug: 4 were on corticosteroids plus cytotoxic drugs and 2 were on hydroxychloroquine plus corticosteroids.

Assessment of disease activity was done using 2 methods. The first was by classifying patients according to the American College of Rheumatology criteria. In this classification patients are divided into 2 groups, those who met 4 criteria out of 11 were considered to have mild disease. Patients who had ≥ 4 criteria were considered to have severe disease.

The second method involved using a standard SLE Disease Activity Index (SLE-

DAI) chart [4]. Assessment was done at the time of the initial examination. A full score for a particular attribute was given when the patient's clinical presentation met the terms for that attribute.

Healthy volunteers were recruited from the relatives of patients. None had a history of major illness and there were no abnormal physical findings during examination or investigations. They were matched for age and sex. Owing to restraints of time and funding, only 30 people were recruited. The same exclusion criteria as for the study group applied and informed consent was obtained.

After an overnight fast, each participant received a single oral dose of 100 mg dapsone (Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt). Drinking of caffeine-containing beverages was not allowed during the study period. A 5 mL blood sample was obtained by venepuncture 3 hours after drug intake and transferred to a 10 mL polyethylene tube containing 50 μ L of heparin (Heparin Leo 5000 IU/mL, Leo Pharmaceutical Products, Ballerup, Denmark). Plasma was separated within 1 hour of collection by centrifugation at 3000 rpm for 10 minutes. The samples were stored at -20°C pending analysis.

A rapid, simple, 1-stage protein precipitation method was used for the estimation of plasma dapsone (DDS) and monoacetyldapsone (MADDS) concentrations by high performance liquid chromatography (Shimadzu Corporation, Kyoto, Japan) [2,5].

Statistical analyses were done using SPSS, version 10. Differences between groups were assessed by the chi-squared test. An estimate was considered to be statistically significant if P -value was < 0.05 . Linear correlation was tested by simple regression analysis [6].

Results

The SLE patient ages ranged from 17 years to 54 years, mean 33.1 years [standard deviation (SD) 10.3]. The group included 42 females (84.0%), age range 17–54 years, with a mean age of 34.4 years (SD 12.5) and 8 males, age range 19–34 years, with a mean age of 28.3 years (SD 8.2).

The ages of the control group ranged from 16 years to 52 years, mean 26.3 years (SD 9.8). The group included 9 males and 21 females (70.0%).

In the SLE patient group, 39 (78.0%) were slow acetylators (MADDS/DDS ratios < 0.30) (Table 1). The slow acetylators included 31 females (79.5%) and 8 males (20.5%) with ages ranging from 17 to 54 years (Table 2). The plasma MADDS/DDS ratios of slow acetylators ranged from 0.01 to 0.28 (mean 0.10; SD 0.082).

Six of the 50 SLE patients, all females, were rapid acetylators (MADDS/DDS ratios > 0.35). Their ages ranged from 18 to 49 years (mean 35.8 years; SD 11.6) (Tables 1 and 2). The plasma MADDS/DDS ratios

Table 1 Frequency distribution of acetylator phenotype in healthy controls and patients with systemic lupus erythematosus (SLE)

Acetylator phenotype	Control group		SLE patients		Total
	No.	%	No.	%	
Rapid ^a	8	26.7	6	12.0	14
Slow ^b	22 ^c	73.3	39 ^d	78.0	61
Non-	0	–	4	8.0	4
Total	30	100	49 ^e	100	79

$\chi^2 4.73$; $P = 0.94$.

^aPlasma dapsone/monoacetyldapsone ratio > 0.35 .

^bPlasma dapsone/monoacetyldapsone ratio < 0.30 .

^c95% confidence interval: 59.9–86.7.

^d95% confidence interval: 63.6–92.4.

^eOne patient was classed as indeterminate.

Table 2 Frequency distribution of acetylator phenotype according to sex in healthy controls and patients with systemic lupus erythematosus (SLE)

Acetylator phenotype	Control group				SLE patients			
	Males		Females		Males		Females	
	No.	%	No.	%	No.	%	No.	%
Rapid ^a	4	50.0	4	50.0	0	–	6	100
Slow ^b	5	22.7	17	77.3	8	20.5	31	79.5
Non-	0	–	0	–	0	–	4	100

^aPlasma dapsone/monoacetyldapsone ratio > 0.35.

^bPlasma dapsone/monoacetyldapsone ratio < 0.30.

ranged from 0.36 to 1.61 (mean 0.81; SD 0.57). One patient with an acetylation ratio of 0.30–0.35 was considered an indeterminate acetylator.

Four of the SLE patients were non-acetylators (MADDS/DDS ratio 0.0). They were all females, aged 23–47 years (mean 31.8 years; SD 11.4) (Tables 1 and 2).

In the control group, there were 22 (73.3%) slow acetylators (Table 1). They included 17 females (77.3%) and 5 males (22.7%) with age range 16–52 years, (mean 27.9 years; SD 10.7) (Table 2). Their plasma MADDS/DDS ratios were 0.01–0.28 (mean 0.11; SD 0.09). There were 8 rapid acetylators in the control group (Table 1). They included 4 females and 4 males (Table 2). The age range was 16–29 years (mean 22.0 years; SD 4.9). Plasma MADDS/DDS ratios were 0.36–1.63 (mean 0.86; SD 0.6). There were no non-acetylators in this group (Table 1).

Analysis of the results showed that SLE patients as a group were not significantly different from the control group regarding the incidence of slow and rapid acetylator phenotypes ($P > 0.05$).

Using the Pearson correlation, no statistically significant correlation was found between plasma acetylation (MADDS/DDS) ratios and American college of rheumatology criteria ($r = 0.058$; $P = 0.700$) in the 50 patients. In addition, the correlation be-

tween plasma acetylation (MADDS/DDS) ratio and the SLE disease activity index in SLE patients was not significant.

Discussion

In this study, the frequency of the slow acetylators in patients with spontaneous SLE did not differ significantly from that in the control group. Godeau et al. were the first to observe an increase in slow acetylators in patients with SLE in 1973 [7]. The association of spontaneous systemic lupus erythematosus (SLE) with slow acetylator status is, however, controversial. While some reports have confirmed the association [8,9], others failed to find any relationship [10,11].

Our findings agreed with results published from other institutions worldwide which showed that slow acetylators are not at greater risk of developing SLE than are those who are rapid acetylators [8,10,11]. Nor do our findings support the notion of a significant association between acetylator phenotype and SLE [9]. Thus in a population with a high percentage of slow acetylators, the phenotype distribution alone seems not to be the contributing factor to the development of spontaneous SLE. This is in agreement with a previous report which failed to

find an association in a predominantly rapid acetylator Chinese population [11].

More recent studies have determined the arylamine N-acetyltransferase genotype of SLE patients. These studies also failed to find an association between the slow acetylation genotype and SLE [12,13].

An interesting finding in our study was that some SLE patients were non-acetylators. In a previous report, about half the patients with Behçet disease were found to be non-acetylators [2]. This finding cannot be explained by a technical error in the method used since non-acetylators in this study as well as the previous one were found in patients and not in the control group. On both occasions patient and control samples were run together. The significance of this finding needs further investigation to determine the genotype of non-acetylators in order to understand this phenomenon.

In previous studies no attempt was made to correlate acetylator status with the severity of the disease. The severity of the disease as measured by both the American College of Rheumatology criteria and the SLE disease activity index did not show any correlation with the acetylator phenotype. This finding is in contrast to a previous report on Behçet disease, another systemic autoimmune disease, which showed a correlation between the severity of the disease and the acetylator phenotype [2].

In conclusion, in a population of slow acetylators, it appears that the slow acetylator phenotype cannot be considered a genetic trait predisposing to the development of spontaneous SLE. In addition, acetylator phenotype does not appear to influence disease activity. The occurrence of non-acetylators does, however, need further investigation.

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