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Original Article

Predictive Value of Gelatin Particle Agglutination Test (GPAT) in Leprosy Detection

TH Khang[1], LT Thanh[2], PH Lanh[3]

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Detection of *Mycobacterium leprae* infection prior to the onset of the clinical disease may be very important for epidemiological study of leprosy and its eradication. By adding diagnosis through early recognition, early treatment can be commenced, thus it would greatly help in the limitation of transmission of *M. leprae* and in reduction of the degree of deformities. The aims of our study was to evaluate the value of GPAT using semi-synthetic trisaccharides antigen (NT-P-BSA, manufactured by FUJIREBIO-INC-Japan and provided by WHO) in early detection of leprosy. 1,030 apparently normal persons including 680 household contacts of patients of leprosy with average 3 years of contact and 350 people living in areas free of leprosy were tested with GPAT. Among 135 household contacts showing positive GPAT, 17 developed leprosy (12.8%) within 14-57 months of becoming positive while among 884 people with GPAT negative, none (0%) developed the disease during the same period of follow-up. In children, a high titer of antibodies constitutes a valuable indicator of high risk in developing the disease: 63.1% of them developed clinical leprosy while it was only 7.7% in adults. 100% leprosy children showing GPAT positive at serum dilution of 1:64 or over have developed leprosy. In conclusion, GPAT has shown that children with GPAT positive at high titer of 1:64 or over and whose mother/father being a leprosy patient can be considered as the highest risk group of eventually developing the disease. All household contacts with GPAT high positivity have been actively followed-up until now. In this sense, GPAT has proved to be an indicator for detection of sub-clinical leprosy infection with high chances of developing clinical disease.

Keywords : Leprosy, Sera, Antigen, *Mycobacterium leprae*, Clinical, Subclinical, Vietnam

Introduction

Early detection of *Mycobacterium leprae* infection prior to the onset of the clinical disease may be very important for epidemiological study of leprosy and its eradication. By adding diagnosis through early recognition, early treatment can be commenced, thus it would greatly help in the limitation of transmission of *M. leprae* and in reduction of the degree of deformity (Surasak et al 1989, Chanteau et al 1987). For this purpose,

[1] Dr. Tran Hau Khang
[2] Dr. Le Trang Thanh
[3] Dr. Pham Hong Lanh
National Hospital of Dermatology and Venereology, Hanoi, Vietnam
Correspondence: Dr. Tran Hau Khang **e-mail**: khangquocduc@fpt.vn

various serological methods using Phenolic Glycolipid I (PGLI), an *M. leprae* specific antigen for the detection of leprosy infection have been used in endemic countries (Brett et al 1983, Dharmendra 1982, and Izumi et al 1990).

In Vietnam, from 1989 onwards, GPAT using semi-synthetic trisaccharides antigen (NT-P-BSA, manufactured by FUJIREBIO-INC-Japan and provided by WHO) has been applied to 1030 apparently normal persons including 680 household contacts of leprosy and 350 people living in areas free of leprosy.

In this paper, we present our results from the continuing prospective study from 1989 to 2016 on the proportion of GPAT positivity in household contacts of leprosy patients and the development of the disease in relation to their age and reactivity of the test.

Materials and Methods

Study subjects : This study included household contacts of leprosy cases as well as controls :

(i) Household contacts : This group comprised of 680 people, who are parents, grandparents, sons/daughters, grandchild, sister/brother, wife/husband... of the patients. They have been in close contact with 127 multibacillary (MB) and 11 paucibacillary (PB) patients for an average period of 3 years (minimum 9.3 months and maximum 4.2 years). All household contacts were screened by clinical examination. All source MB/PB patients (index cases) were classified based on a bacterial index (BI) and clinical manifestation. They were treated with MDT according to the WHO recommendation.

(ii) Control group : This group included 350 apparently health persons without known contact with leprosy patients and living in areas exempt of leprosy.

Ethical statement : The study was approved by the Ethical Committee of the National Hospital of Dermatology and Venereology. All participants were volunteers, they signed an informed consent form. Parents or tutors signed the form for children.

Sera : Sera from controls and household contacts were collected when index source cases were detected and then stored in ice bottles and transferred to the central laboratory of the National Hospital of Dermato-Venereology in Hanoi within the day of their collection.

Antigen : Antigen used was trisaccharide-BSA conjugate carrying the P-(2-Methoxycarbynylethyl) phenyl group as a linker arm (NT-P-BSA) (manufactured by FUJIRIBEO-INC-Japan, and provided by WHO).

Method : The technique used was as described in the instruction sheet enclosed in the GPAT kits.

It may, however, be noted that positive specimen in the qualitative assay using different serum dilution may also be used as an alternate to confirmatory tests. Serum dilution of 1:32 and over is considered as a criterion of positivity. Agglutination that appears at the serum dilution of 1:16 or lower was labeled as negative.

Analysis was performed using statistical software (SPSS version 13; SPSS Inc, Chicago, Ill). All tests of significance were 2-sided, and statistical significance was assumed at $p < 0.05$.

Results

GPAT in normal controls : Among 350 control assayed, 11 (3.1%) were GPAT positive. The proportion of positivity of GPAT was highest (but not statistically significant) at the age ranging from 15 to 29 years (4.2%) (Table 1).

GPAT in household contacts : Out of 680 household contacts, 135 were GPAT positive, showing a proportion of 20% of positivity. (Table 2)

Table 1 : GPAT in normal controls

Age	No assayed	GPAT positive	
		No	%
<15	78	2	2.5
15-29	95	4	4.2
30-39	80	3	3.8
40-49	40	1	2.5
50-59	45	1	2.2
>60	12	0	0.0
	350	11	3.1

Table 2 : GPAT in household contacts classification according to age group

Age	No assayed	GPAT positive	
		No	%
<15	264	45	17.0
15-29	126	27	21.4
30-39	117	28	24.8
40-49	84	18	21.4
50-59	45	9	20
>60	44	8	18.2
	680	135	20.0

As shown in Table 2, the proportion of cases with GPAT positive reached the highest value at the age group of 30-39 years (24.8%). There was no statistically significant correlation between the reactivity of GPAT test and the types of blood relationship to patients as shown in Table 3.

Development of clinical leprosy among GPAT positive cases :

- According to age group shown in table 2, out of 680 household contacts, 264 were children (< 15 years old) and 416 adults (> 15 years old).
- Among 264 children, 45 were GPAT positive (17%). In adults, this proportion was 21.6% (90/416), thus, no significant difference as regard to seropositivity proportion was found between children and adults (P>0.2).
- After 5 years of follow up (1989-1994), out of 45 children with GPAT positive, 12 developed leprosy (26.7%) while among 90 adults who were GPAT positive, only 5 have developed the disease (5.6%). All positive household contacts have been actively followed-up, but no more new cases were detected since then up to now (2016).

Table 4 showed the relation between the disease development and the serum dilution in subjects with GPAT positive. Among 19 children with GPAT positive at the serum dilution of 1:64 and over, 12 have developed leprosy while none has

Table 3 : GPAT in household contacts classification according to blood relationship with patients

Relationship with patients	No assayed	GPAT positive	
		No	%
Child	189	34	18.0
Parent	48	10	20
Grandfather/grandmother	89	16	18
Brother/sister	103	21	20.4
Wife/husband	162	39	24.1
Other	89	15	17.0
	680	135	20

Table 4 : Household contacts with GPAT positive developing clinical leprosy

Age	Number		Serum dilution							
			1:32		1:64		1:128		1:256	
	GPAT positive	Development of leprosy	GPAT positive	Development of leprosy	GPAT positive	Development of leprosy	GPAT positive	Development of leprosy	GPAT positive	Development of leprosy
<15	45	12	26	0	13	8	5	3	1	1
>15	90	5	51	2	36	2	3	1	0	0
	135	17	77	2	49	10	8	4	1	1

developed the disease among 26 children with seropositivity at serum dilution of 1:32.

Concerning blood relationship, as shown in Table 5, all 12 children developing leprosy (100%) had their mother/father suffering from leprosy. One 9 year old child whose GPAT test was strongly positive at the serum dilution of 1:256 has developed LL type of leprosy with BI=6+. Duration to develop positivity ranged from 14 -57 months after detecting positivity.

Discussion

Subclinical infection with *M. leprae* has recently been considered as one of the most important problem in leprosy control and research (Gonzalez et al 1990, Stefani et al 1988). Various serological methods using PGL-I have widely been used in some leprosy epidemic countries aiming at gaining better understanding of the epidemiological trends and finding the individuals who are at high risk of developing the disease (Izumi et al 1990, Menzel et al 1987, Tsuyoshi and Shinzo 1987). Studies showed elevated levels of antibodies to PGL-I in household contacts of leprosy (Roche et al 1999, Soares et al 1994). Could it be considered as an indicator for early diagnosis of leprosy ?, is difficult to answer. As presented above, our results showed that 135 out of 680 (20%) household contacts were GPAT positive at serum titer of 1:32 – 1:256 while among 350 controls, only 11 were of weak seropositivity at serum dilution of 1:32 (3.1%). This indicates that contacts had more chance of being infected by leprosy bacilli and this was true for all groups of ages. Moreover, the controls

Table 5 : Leprosy type in relation to age, serum titer, BI, duration and blood relationship to index patients and their type

No	Clinical leprosy newly developed	Age	Type of leprosy	BI	Serum titer	Relationship to patient and type of source case	Duration to develop leprosy after positivity
1.	NDP	12	I	0	1:64	F BB, BI:2+	14 months
2.	BTD	8	TT	0	1:64	F BL, BI:2+	32 months
3.	NVS	12	I	0	1:64	F BL, BI:3+	29 months
4.	NVT	11	BT	0	1:128	M BB, BI:2+	17 months
5.	NVT	14	I	0	1:64	F BL, BI:4+	32 months
6.	BDT	10	BT	0	1:128	F+M TT, BI: Neg BB, BI:2+	19 months
7.	VTT	14	I	0	1:64	F BL, BI:3+	26 months
8.	CTV	10	I	0	1:64	F+M BT, BI:Neg BB, BI:3+	23 months
9.	CTM	13	I	0	1:128	F LL, BI:5+	15 months
10.	TMH	13	BT	0	1:64	F+M TT, BI:Neg BB, BI:2+	19 months
11.	LTH	10	TT	0	1:64	F+M BT, BI: Neg BB, BI:2+	23 months
12.	LVV	9	LL	6+	1:256	F+M BT, BI:Neg LL, BI: 5+	17 months
13.	DVC	18	TT	0	1:64	M BL, BI: 4+	21 months
14.	HTN	17	I	0	1:128	F BB, BI: 3+	14 months
15.	NV.T	37	TT	0	1:32	Brother BL, BI: 4+	36 months
16.	NTO	31	TT	0	:64	Sister BB, BI: 3+	56 months
17.	TVT	26	BT	0	1:32	Relative BL, BI:4+	57 months

Abbreviations : F: Father, M: Mother, Neg: Negative

living in areas free of leprosy with GPAT positive seemed to have contacted patients in somewhere.

It is known that among contacts, only a small proportion of those exposed to *M. leprae* will develop leprosy. One of the purposes of our study was to attempt to define individuals at high risk of developing the disease. We think that positivity of GPAT could be considered as an indicator of high risk group people. As a matter of fact, 17 among 135 household contacts with GPAT positive have developed leprosy while none of 884 subjects with GPAT negative have developed the disease during the same period of follow-up.

The diagnosis and classification of newly developed leprosy cases among GPAT tested people was based on clinical, bacteriological and/or histopathological features. Among 17 cases, 16 belong to PB (I, TT, BT) and only one with very strong seropositivity was MB (LL). All these new patients were treated with MDT/WHO while all the contacts with strong positivity up to 1:64 and 1:128 were actively followed every 3 months to detect the disease.

Interestingly, there was a clear correlation between the age group and the disease development. Indeed, out of 45 children with seropositivity, 12 have developed leprosy (26.7%) while in adults, this proportion was only 5.5%. Thus, chi-square test was statistically significant when comparing the proportion of disease development in children and in adults ($P<0.002$; $X2=8.9$). More interesting is the relation between the disease development in subjects with GPAT positive and their serum titer: out of 19 children with GPAT positive at serum titer of 1:64 or over, 12 have developed leprosy (63.1%) while among 26 with GPAT positive at a low titer (1:32), none has developed the disease. As regard to blood relationship to patient, it must be emphasized that all these 12 cases were in close contact with their mother/father who were leprosy patients.

In recent years, several studies have shown that the seropositivity rate was significantly higher among those contacts living in households where new cases emerged than among the contacts living in households where no new cases were detected. (Cardona et al 2008 and Douglas et al 2004). Apart from GPAT, several tests such as Fluorescent Leprosy Antibody Absorption (FLA-ABS), ELISA etc have been carried out for detection of subclinical infection in leprosy. In India, in 8years of follow-up, 46 contacts have developed disease and of these 41 contacts were FLA-ABS positive (Bharadwaj and Katoch 1989).

The seroprevalence rates of antibodies to phenolic glycolipid-1 among children have also been studied in many countries such as the Philippines, India, Colombia, Thailand… and it was considered as an indicator of leprosy endemicity status and such individuals were found to be at higher risk of developing leprosy. (Stella et al 1999, Nora et al 2005, Surasak et al 1989). Our study shows a clear correlation between levels of positivity and risk of getting disease in next 1-3 years, even though we may not be able to determine when original exposure occurred.

In conclusion, GPAT has shown that children with GPAT positive at high titer of 1:64 or over and whose mother/father being a leprosy patient can be considered as the highest risk group of eventually developing the disease. In this sense, GPAT has proved to be an indicator for early detection of leprosy in children.

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