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Serodiagnosis of Typhoid Fever by Enzyme-Linked Immunosorbent Assay(ELISA)*

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=국문초록=

효소면역측정법에 의한 장티푸스의 혈청학적 진단

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장티푸스가 의심되는 51명의 환자 혈청내에서 *Salmonella typhi*의 균체항원에 대한 IgG, IgM과 IgA 항체를 효소면역측정법으로 측정하였다. 균체항원에 대한 IgG와 IgA 항체가는 세균이 분리되어 장티푸스로 확진된 환자에서 건강대조군보다 높았다. 반면 세균을 분리하지는 못하였지만 임상증상 등으로 장티푸스를 의심하는 환자에서는 IgM항체가는 건강대조군보다 높았다. 균체항원에 대한 항체를 효소면역 측정법으로 측정하는 것이 Widal 검사보다 민감도가 높았다.

장티푸스를 진단하는데 균체항원을 부착시켜 효소면역측정법을 사용하는 것이 유용할 것이다.

Key Words: serodiagnosis, typhoid fever, ELISA

INTRODUCTION

Typhoid fever has been prevalent in many developing countries[7, 10, 12]. It has been important for clinician to diagnose typhoid fever by determination of serum antibodies. However, the Widal test, the most common serological test for the diagnosis of typhoid fever, is insensitive[5, 11, 15]. And in the Widal test, nonspecific reactions are frequently observed and antibodies of immunoglobulin M (IgM) class are preferentially detected[6]. On the other hand, in enzyme-linked immunosorbent assay(ELISA), very small amount of antibodies can be separately measured. The purpose of this study is to measure the IgG, IgM and IgA antibody in sera from clinically suspected typhoid fever patients and healthy control by ELISA in order to determine whether ELISA values can be clinically applied to diagnose the typhoid fever.

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MATERIALS and METHODS

1. Grouping of subjects studied

Fifty-one patients admitted in Seoul National University Hospital with clinically suspected typhoid fever were categorized into group II to V according to the results of blood culture and the Widal test as shown in Table 1. The titer above 1 : 80 was regarded as positive cut-off titer in the Widal test because in endemic area such as the developing countries, there were some healthy persons with antibody titer higher than 1 : 80 in the Widal test[11, 13, 14, 15]. Among these, all were not vaccinated prior to admission, except for 8 patie-

Table 1. Characteristics of groups categorized

Group	Symptom	Blood culture	Widal titer*	Patient number
I	−	N.D.**	L	25
II	+	+	H	14
III	+	+	L	16
IV	+	−	H	9
V	+	−	L	12

*L : low agglutination titer (≦ 1 : 80)
H : high agglutination titer (≧ 1 : 160)
**N.D. : not done

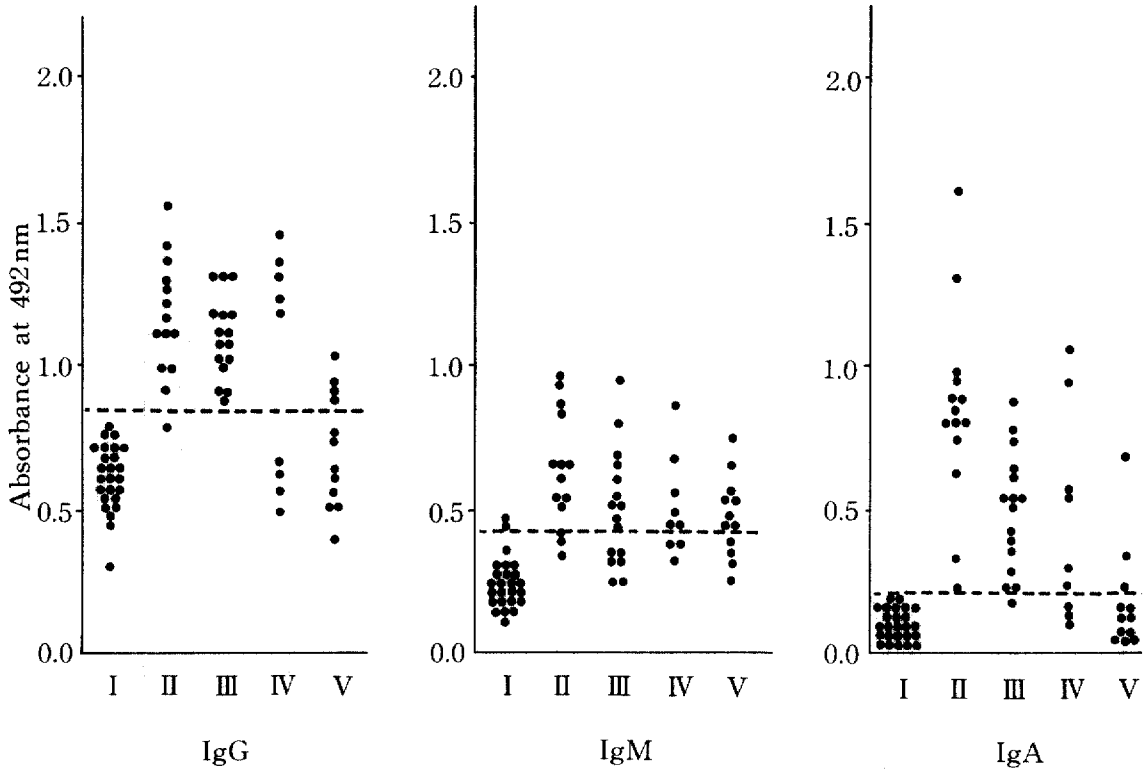

Fig. 1. Antibody to the heat-Rilled whole bacterial antigen of *Salmonella typi* : ELISA absorbance values for serum samples from each group. The dashed lines represent the cut off value (mean+2 standard deviations of the values for healthy control i.e. group I).

nts with uncertain history. Twenty-five healthy medical students without history of typhoid fever and vaccination were categorized into group I as control. They showed antibody titer 1 : 80 or below in the Widal test.

2. Bacterial Strain

Salmonella typhi O901w was used, which was subcultured and preserved in the Department of Microbiology, College of Medicine, Seoul Nation University.

The heat killed whole bacterial antigen (WBA) was prepared according to Garvey et al[9].

3. ELISA

Heat killed WBA of *S. typhi* was used at 10^8 cells/ml in distilled water. Antigen was coated in the well of the polyvinylchloride plates(Flow Lab.) by drying and methanol fixa-

tion according to the method of Briles et al[2]. Further procedures were followed largely by Kye et al[9]. Briefly, serum samples were diluted to 1 : 80 in phosphate buffered saline (pH 7.2) containing 0.1% bovine serum albumin. After 1 hour incuvation at 37℃, peroxidase conjugated antihuman class-specific immunoglobulin (IgG, IgM or IgA, Cappel Lab.) was reacted for 1 hour at 37℃.

Finally substrate containing *o*-phenylenediamine in 0.02 M phosphate citrate buffer (pH 5.0) was reacted at room temperature. Color reaction was measured by ELISA reader (Multiskan, Flow Lab.) at 492 nm.

4. Widal test

Titration of anti-*S. typhi* O agglutinins was performed by the standard tube method.

RESULTS

The absorbance values were obtained with the sera from each group for IgG, IgM and IgA anti-WBA of *S. typhi* as illustrated in Fig. 1.

The IgG ELISA values of the sera from the control group revealed wide range of absorbance from 0.31 to 0.77, but those of the sera from the culture positive patients (group II + III) revealed higher ones than 0.77. Those of about half of the sera from culture-negative patients (group IV + V) revealed lower ones than 0.77. The IgM ELISA values were variable: the values of the sera from patient groups were indistingulshable from those from the control. Overall distribution of IgA ELISA values was similar to that of IgG values, but the IgA ELISA values of the control were quite low.

The means and standard deviation of IgG, IgM and IgA values for the control group were 0.62 ± 0.11, 0.26 ± 0.08 and 0.08 ± 0.06, respectively. With the mean plus 2 standard deviations as the cut-off values, serum samples with an absorbance of more than 0.84, 0.42 and 0.20 were defined as positive for IgG, IgM and IgA anti-WBA of *S. typhi* respectively. The blood culture-positive groups (group II + III) revealed high positive rate for the IgG and IgA antibodies in ELISA. The IgM ELISA value was considered to be inappropriate, as more than one-fourth the culture-positive groups were negative for the IgM antibody according to the definition. As shown in Table 2, the positive rate for IgG, IgM and IgA anti-WBA of *S. typhi* is 96.7%, 70.0% and 96.7 %, respectively. In the culture-negative groups (group IV + V), the positive rate for IgG, IgM and IgA antibodies is 42.9%, 66.7% and 42.9 %, respectively. Comparing to the Widal test, there was no statistically significant difference in the positive rate for each antibody in ELISA as the positive rate of group II and group IV were statistically indistingushable from

Table 2. Results of the ELISA for immunoglobulin anti-*S. typhi* whole bacterial antigen

Serum source	No. of samples	No.(%) of serum samples positive for immunoglobulin anti-*S. typhi* whole bacterial antigen		
		IgG	IgM	IgA
Group I	25	0	2(8.0)	0
Group II	14	13(92.9)	11(78.6)	14(100)
Group III	16	16(100)	10(62.5)	15(93.8)
Group IV	9	5(55.6)	6(66.7)	6(66.7)
Group V	12	4(33.3)	8(66.7)	3(25.0)
Group II + III	30	29(96.7)	21(70.0)	29(96.7)
Group IV + V	21	9(42.9)	14(66.7)	9(42.9)
Group II + IV	23	18(78.3)	17(73.9)	20(87.0)
Group III + V	28	20(71.4)	18(64.3)	18(64.3)
Group II + III + IV + V	51	38(74.5)	35(68.6)	38(74.5)

that of group III and group V, respectively, except for IgA values between group IV and group V.

As an overall view, it seems that the positive rate is slightly higher in the Widal test-positive groups than in the Widal test-negative groups.

DISCUSSION

Enzyme-linked immunosorbent assay (ELISA) has some advantages; for example, detectability of very small amount of antigen or antibody, separate measurement of each class of immunoglobulin and high reproducibility[4]. ELISA has been used to measure the antibody against *S. typhi* antigens. Carlsson et al[1,3], detected IgG and IgM antibodies against *S. typhi* lipopolysaccharide by ELISA. Beasley et al[1] reported the similar results by using cell envelop farction antigen. Kye et al[9], used whole bacterial cell as an antigen.

In this study IgG, IgM and IgA antibodies to whole bacterial antigen of *S. typhi* were detectable by ELISA; more sensitively IgG and IgA antibodies. Whereas the Widal test was rather crude immunological method largely involving IgM antibody[6].

The sensitivity of the Widal test was quite low, as the positivity of the culture-positive and negative patient groups was 46.7% and 42.9%, respectively. On the other hand, in ELISA, the positive rate for IgG and IgA antibodies was 96.7% in the culture-positive group and 42.9% in the culture-negative group as shown in the Table 2. This fact represented that ELISA was a tool superior to the Widal test in the diagnosis of the actual patients of typhoid fever.

In culture-negative patients groups, the positive rate for IgM antibody was significantly high as compared with that of IgG and IgA antibodies suggesting that the IgM ELISA value was useful to diagnose the unproven case of typhoid fever. The positive rate for IgM antibody by ELISA was also significantly superior to that by the Widal test.

Sera from one *Escherichia coli* bacteremia patient, two shigellosis patients and two rheumatoid arthritis patients showed all negative ELISA values in all classes of antibody (Data not shown). This data was not sufficient to provide a definite conclusion of specificity and it would be neccessary to study ELISA values of sera of many patients with bacteremia from non-typhoid strains of *Salmonella*.

In conclusion this assay would be very useful for clinician to diagnose the typhoid fever.

SUMMARY

Serum samples from 51 patients with clinically suspected typhoid fever were tested for immunoglobulin G (IgG), IgM and IgA antibodies against the whole bacteria antigen of *Salmonella typhi* by an enzyme-linked immunosorbent assay. The levels of IgG and IgA antibody to-whole bacteria antigen were higher in the culture-proven patients than in controls. The levels of IgM antibody to-whole bacteria antigen showed better discrimination between culture negative patients and controls than those of IgG or IgA antibody to-whole bacteria antigen. The enzyme-linked immunosorbent assay was much more sensitive than the Widal test. It would be a useful tool for the diagnosis of typhoid fever with a single serum sample.

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