The Comparison of Widal Titer in Healthy Individuals Living in Good and Poor Sanitation Environment in Langsa City, Aceh Province, Indonesia

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Abstract: Widal test is one of the diagnostic tools used to establish typhoid fever. However, the test may show false positive particularly among healthy individuals living in a poor sanitation environment. The aim of this study is to compare the titer of Widal test among healthy individuals living with good or poor sanitation. A total of 180 healthy individuals resided in Langsa City were enrolled in the study between March and May 2018. Sanitation status of each individual was recorded, assessed and classified into good or poor sanitation. Of these, 90 and 90 individuals were defined as having good and poor sanitation, respectively. The proportion of positive Widal titer on Salmonella typhi O was 66.7% (60/90) in individuals with good sanitation and 61.1% (55/90) among individuals with poor sanitation (P>0.05). However, conversion of *S. Typhi* H was more frequent among healthy individuals living with poor sanitation (OR 3.348, 95 % CI = 1·754-6.390). We conclude that the sanitation level increased the antibody titers in the Widal test, and therefore Widal test has limited use to diagnose typhoid fever. Further study is needed to evaluate behavioral risk factors associated with increased Widal titer and the cut-off level for Widal titer in population in Langsa City.

1 INTRODUCTION

One of the most common infectious diseases in developing countries is typhoid fever. In 2003, the World Health Organization (WHO) estimated around 17 million cases of typhoid fever worldwide with an incidence of 600.000 deaths every year. Based on data from Basic Health Research in Indonesia (RISKESDAS) in 2007, the prevalence of typhoid fever in Indonesia reached 1.7%. However, there is no report about typhoid prevalence in RISKESDAS 2013. The highest prevalence was in children aged 5-14 years (1.9%), followed by aged 1-4 years (1.6%), 15-24 years (1.5%) and ages less than 1 year (0.8%). According to the WHO data published in 2014 it is estimated that there are around 21 million cases of typhoid fever worldwide with death reaching 222 thousand people (Masitoh 2009; Elisabeth 2016; WHO 2014). Data from Langsa City General Hospital showed that from 2016 to 2018, typhoid fever was one of the top ten diseases in hospitalized patients, which were 902(11%), 801(12%), and 1113(15%) cases, respectively (Langsa City General Hospital Profile 2016-2018).

Typhoid fever is an acute systemic infectious disease caused by gram-negative bacteria *Salmonella enterica serotype Typhi* (*Salmonella typhi*), a quick and precise diagnosis is needed as early as possible in suspected patients having typhoid fever in order to get the right treatment immediately. Widal test is a modality which is often used to diagnose typhoid fever (Putri Satwika 2016) due to an easy, inexpensive, and relatively noninvasive procedure which can be used as a diagnostic value where blood culture is not available (Alam et al 2011). The Widal test diagnostic value is to address a significant increase of antibody titers in blood against O (somatic) and H (flagellar) antigen *S. typhi*. However, Widal test has a low

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sensitivity and specificity, also have limitations in interpreting the results, with many false positive and false negative results due to the prevalence of basic healthy titers in certain endemic or geographical regions and sanitary conditions (Wardana et al 2014; Jemilohun 2017; Chauhan 2016).

Data from the Langsa City Health Profile in 2014 reported one of the indicators of good environmental condition was community-based total sanitation (STBM). The number of villages implementing STBM in Langsa Barat District is 100%, while in Langsa Timur District 37.5% (Langsa City Public Health Department, 2014). Seeing the indicators of environmental conditions that have not met the requirements, it will be more likely the healthy person will have positive results, thus when the person had a fever, diagnostic errors often occur (false positive). Therefore we want to assess the difference in Widal titers of healthy individuals who live in good sanitation compared to in poor sanitation environments.

2 METHODS

This was an analytic observational study with a cross-sectional design. This study was conducted in Langsa City, located in Langsa Timur and Langsa Barat District, from March to May 2018. The samples were healthy individuals aged >18 years who lived in environments with good sanitation characteristics in Langsa Barat district and poor sanitation characteristics in Langsa Timur district. The initial assessment used an observation sheet on basic environmental sanitation according to the Minister of Health Decree No. 829 / Menkes / SK / VII / 1999, covering assessment including clean water facilities, latrines (sewage disposal facilities), wastewater disposal facilities and waste disposal facilities, with a total assessment of > or = 334 categorized as good sanitation criteria and <334 as a poor sanitation criteria. A total sample of 180 people was enrolled. Data collection used purposive sampling method. Populations that meet the inclusion criteria are subjects in good health, not suffering fever (normal body temperature 36.5ºC - 37.2ºC), aged >18 years and residing in Langsa Barat and Langsa Timur District. While the exclusion criteria were subjects who were not willing to take blood samples and participate in the study.

Widal serological tests were performed on blood samples from all study subjects to observe the occurring agglutination (Agarwal et al 2016), using the "AIM" brand reagents which consisted of; Antigen *S. typhi* H, *S. paratyphi* AH Antigen, *S. paratyphi* BH Antigen, *S. paratyphi* CH Antigen, *S. Typhi* O Antigen, *S. paratyphi* AO Antigen, *S. paratyphi* BO Antigen and *S. paratyphi* CO Antigen. All samples were taken directly to the Langsa City General Hospital Laboratory for examination. The procedure was carried out in accordance with the Standard Operating Procedure of the Widal examination at the Langsa City General Hospital Laboratory.

Data processing using Statistical Package for the Social Science (SPSS) version 22.0 was presented descriptively to see the proportion of Widal titers of healthy individuals in good environmental sanitation and poor environmental sanitation. Analysis using Chi-Square Test and Odds Ratio with significance level $p < 0.05$ and 95% confidence interval to assess the relationship of risk factors with positive Widal results.

This study has been approved by the Ethics Committee of Faculty of Medicine, Universitas Sumatera Utara with ethical clearance No. 158 / DATE / KEPK FK USU-RSUP HAM / 2018.

3 RESULTS

There was no difference in the age group between the two study groups. Most of the participants were educated until high school in both groups, 38 people (42.2%) in the poor sanitation group and 48 (53.3%) in the good sanitation group (see Table 1).

Table 1: Demographic characteristic of research subjects.

Demographic Characteristic	**Poor Sanitation (=90)**	**Good Sanitation (n=90)**
Sex, n (%)		
Man	28 (31.1)	33 (36.7)
Woman	62 (68.9)	57 (63.3)
Age, n (%)		
< 20 year	3 (3.3)	1 (1.1)
20-29 year	17 (18.9)	20 (22.2)
30-39 year	28 (31.1)	20 (22.2)
40-49 year	16 (17.8)	17 (18.9)
50-59 year	16 (17.8)	21 (23.3)
≥ 60 year	10 (11.1)	11 (12.2)
Education, n (%)		
Uneducated	4 (4.4)	0
PS* undergraduate	6 (6.7)	4 (4.4)
PS	11 (12.2)	6 (6.7)
JHS**	22 (24.4)	2 (2.2)
SHS***	38 (42.2)	29 (32.2)

University	9 (10)	48 (53.3)
Occupation, n (%)		
Housewife	40 (44.4)	19 (21.1)
Unemployed	2(2.2)	3 (3.3)
Entrepreneur	13 (14.1)	30 (33.4)
Student	4 (4.4)	4 (4.4)
Farmer	26 (28.9)	0
Civil Servant	5 (5.6)	34 (37.8)

*primary school, **junior high school, ***senior high school

Conversion for Widal test was most occurred for *S. Typhi O* in 115 people (63.9%), followed by conversion for *S. Typhi H* in 64 people (35.6%). While the smallest numbers of positive results in Widal was for agglutinin *S. paratyphi* 35 people (19.4%) (see Table 2).

Table 2 Widal test result from overall research subjects.

Agglutinin	**Positive (>1/80)**	**Negative (< or = 1/80)**
S.thypi O, n (%)	115 (63.9)	65 (36.1)
S.parathypi AO, n (%)	57 (31.7)	123 (68.3)
S.parathypi BO, n (%)	60 (33.3)	120 (66.7)
S.parathypi CO, n (%)	47 (26.1)	133 (73.9)
S.thypi H, n (%)	64 (35.6)	116 (64.4)
S.parathypi A H, n (%)	35 (19.4)	145 (80.6)
S.parathypi B H, n (%)	36 (20)	144 (80)
S.parathypi CH, n (%)	37 (20.6)	143 (79.4)

According to the study group, the conversion for Widal test in the good sanitation group was for *S. thypi O* as many as 60 people (66.7%), followed by *S. parathypi* agglutinin *AO* as many as 33 people (36.7%) The smallest positive results were 13 people (14.4%) on agglutinin *S. paratyphi B H* (see Table 3).

Table 3 Comparison of Healthy Individual Widal Titer in Good Sanitation Environment in Langsa City.

Agglutinin	**Positive (> 1/80)**	**Negative (< or =1/80)**
S.typhi O	60 (66.7)	30 (33.3)
S.paratyphi A O	33 (36.7)	57 (63.3)
S.paratyphi B O	30 (33.3)	60 (66.7)
S.paratyphi C O	32 (35.6)	58 (64.4)
S.typhi H	20 (22.2)	70 (77.8)
S.paratyphi A H	15 (16.7)	75 (83.3)
S.paratyphi B H	13 (14.4)	77 (85.6)
S.paratyphi C H	16 (17.8)	74 (82.2)

While in the poor sanitation group, the agglutinin conversion was more likely to occur in *S. Typhi O* (55 people, 61.1%), followed by agglutinin *S. typhi H* as many as 44 people (48.9%), and the least likely to occur in agglutinin *S. Paratyphi CO* in 15 people (16.7%) on *(*see Table 4).

Table 4 Comparison of Healthy Widal Individual Titer in Poor Sanitation Environment in Langsa City.

Agglutinin	Positif (> 1/80)	Negative (< atau = 1/80)
S.typhi O	55 (61.1)	35 (38.9)
S.paratyphi A O	24 (26.7)	66 (73.3)
S.paratyphi B O	30 (33.3)	60 (66.7)
S.paratyphi C O	15 (16.7)	75(83.3)
S.typhi H	44 (48.9)	46 (51.1)
S.paratyphi A H	20 (22.2)	70 (77.8)
S.paratyphi B H	23 (25.6)	67 (74.4)
S.paratyphi C H	21 (23.3)	69 (76.7)

In Langsa Barat district, the highest percentage of *S. Typhi O* agglutinin was 1/320 titer in 42 (46%) subjects. Whereas in East Langsa Timur District, *S. Typhi O* agglutinin titers were obtained with the highest percentage was 1/80 titers in 35 (38.9%) subjects (see Table 5).

Analysis of the Widal test results between the subjects who live in the area with good and poor sanitation are presented in table 6. People living in poor sanitation were more likely to show agglutinin *S. typhi* H (OR = 3.348, 95% CI, *P*<0.001)*, S. paratyphi A H* (OR = 1.429, 95% CI, *P*<0.346), *S. paratyphi B H* (OR = 2.033, 95% CI, *P*<0.062), *S. paratyphi C H* (OR = 1.408, 95% CI, *P*<0.356).

Table 5 Percentage of Widal Titer in the Good Sanitation and Poor Sanitation Area in Langsa City.

Agglutinin	Good Sanitation (n=90)			Poor Sanitation (n=90)		
	1/80	1/160	1/320	1/80	1/160	1/320
S. Typhi O, n(%)	30(33.3)	18(20)	42(46.7)	35(38.9)	34(37.8)	21(23.3)
S. paratyphi AO, n(%)	57(63.3)	22(24.4)	11(12.2)	66(73.3)	18(20)	6(6.7)
S. paratyphi BO, n(%)	60(66.7)	20(22.2)	10(11.1)	60(66.7)	25(27.8)	5(5.6)
S. *paratyphi CO*, n(%)	58(64.4)	19(21.1)	13(14.4)	75(83.3)	12(13.3)	3(3.3)
S. *Typhi H*, n(%)	70(77.8)	8(8.9)	12(13.3)	46(51.1)	24(26.7)	20(22.2)
S. *paratyphi AH*, n(%)	75(83.3)	10(11.1)	5(5.6)	70(77.8)	9(10)	11(12.2)
S. *paratyphi BH*, n(%)	77(85.6)	8(8.9)	5(5.6)	67(74.4)	15(16.7)	8(8.9)
S. *paratyphi CH*, n(%)	74(82.2)	11(12.2)	5(5.6)	69(76.7)	11(12.2)	10(11.1)

4 DISCUSSION

Our study showed there is an influence of the level of sanitation on the results of *S. Typhi H*. agglutinin test, where individuals living in poor sanitation was more likely to have positive results compared to individuals living in a good sanitation environment. This result is in line with other studies which have described the results of Widal test rely on sanitary conditions, the prevalence of basic titers of healthy residency in certain endemic and geographical regions (Wardana et al 2014; Jemilohun 2017; Chauhan 2016).

Poor environmental sanitation and health conditions affect the yield of high titers. In addition, several factors such as nutritional condition at the time of test, prior administration of antibiotics, immunological status, vaccination, use of immunosuppressive drugs, cross-reaction with other *Enterobacteriaceae* and Widal test methods used also affect the results. These factors are not further evaluated in this study. The increase in titer of agglutinin H alone without an increase in agglutinin O should not be used to diagnose typhoid fever (Zorgani et al 2014), but may help in diagnosing suspected typhoid fever in adult patients from non-endemic areas or in children less than 10 years old in endemic area, due to the possibility of contact with *S. Typhi* in subinfection doses. Thus, if Widal is still needed to support the diagnosis of typhoid fever, the threshold for referral titers, both in children and adults, needs to be determined (Gaikwad et al 2014).

Widal results on 180 total blood samples from the study subjects showed an agglutination reaction between antibodies with Widal antigen. Widal tests were considered positive if the antibody titer is 1/160 (Loho et al 2000), both for agglutinin O and H with single or combined diagnostic criteria if a single criterion is used. Furthermore, agglutinin O is also found to be more diagnostic than agglutinin H (Zorgani et al 2014). In this study, we found more than 50% of the healthy individuals studied were positive for Widal test (titer>1/80). This is in line with Chauhan's (2016) study in Uttar Pradesh, India that among 250 healthy individuals who were performed Widal test, 56.8 % of them showed a titer of 1/80 for anti-0 and anti-H antibodies, leading to set this titer as the baseline titer for diagnosing typhoid fever (Chauhan 2016). Having known the titer for the conversion of Widal test among our population in Langsa, we set up a titer of 1/80 against antibody H to be used for the diagnosis of typhoid fever.

Based on the results of Widal test of healthy individuals in the two sanitation area groups, the most frequent positive test result was for agglutinin *S. thypi O* in 60 people (66.7%) in good sanitation and in 55 people (61.1%) in poor sanitation. This shows that *Salmonella* agglutinin is generally found in individuals who appear to be healthy and not suffering from fever when having their blood examined in different populations and sanitation. It also concluded that the Widal test is easy to applicate but has limitations in endemic areas, including Indonesia (Suryani et al 2018). Therefore, if the Widal test is still needed to support the diagnosis of typhoid fever, the threshold for reference titers for both children and adults needs to be determined (Zorgani et al 2014).

Table 6 Differences in Widal Test Results Based on Environmental Sanitation Conditions.

Agglutinin	**Poor Sanitation n=90**	**Good Sanitation n=90**	**P**	**OR 95%CI**
S. typhi O				
Positive	55 (61.1)	60 (66.7)	0.438	0.786
Negative	35 (38.9)	30 (33.3)		0.427-1.446
S. paratyphi A O				
Positive	24 (26.7)	33 (36.7)	0.149	0.628
Negative	66 (73.3)	57 (63.3)		0.333-1.184
S. paratyphi B O				
Positive	30 (33.3)	30 (33.3)	1.000	1.000
Negative	60 (66.7)	60 (66.7)		0.538-1.859
S. paratyphi C O				
Positive	15 (16.7)	32 (35.6)	0.004	0.363
Negative	75(83.3)	58 (64.4)		0.180-0.732
S. Typhi H				
Positive	44 (48.9)	20 (22.2)	<0.001	3.348
Negative	46 (51.1)	70 (77.8)		1.754-6.390
S. paratyphi A H				
Positive	20 (22.2)	15 (16.7)	0.346	1.429
Negative	70 (77.8)	75 (83.3)		0.679-3.008
S. paratyphi B H				
Positive	23 (25.6)	13 (14.4)	0.062	2.033
Negative	67 (74.4)	77 (85.6)		0.956-4.325
S. paratyphi C H				
Positive	21 (23.3)	16 (17.8)	0.356	1.408
Negative	69 (76.7)	74 (82.2)		0.679-2.916

Our study also showed the highest value of agglutinin was at 1/320, this was also reported in another study by Bahadur and Peerapur (2013) in healthy individuals in Karnataka, India (Bahadur et al 2013). Thus, if the same Widal titer obtained from patients with suspected typhoid fever who seek treatment at health care facilities in Langsa City, relying on Widal as the only diagnostic laboratory test of typhoid fever will generate a misleading diagnosis, with a false positive possibility. When blood culture is compared to the Widal test for the diagnosis of typhoid fever, the specificity of the Widal test reduced significantly. Of 270 individuals with suspected typhoid fever and positive O and H antibodies, 74.4% were negative in blood culture and only small proportion was positive for *S. Typhi* (N=7, 2.6%) and *S. paratyphi* (N=1.5%) (Andualem, 2014). Therefore, it is very important to do more accurate tests such as culture to confirm typhoid fever.

5 CONCLUSION

This study showed there was an increase in Widal titers of healthy individuals in good and poor sanitation environment in Langsa City. Healthy individuals living in poor sanitation environment were at risk of having 3.348 times positive *S. Typhi* Widal titers compared to healthy individuals living in good sanitation environment. Widal test, therefore, can give a false-positive interpretation in the diagnosis of typhoid fever and should be used with other tools with better specificity. There are limitations in this study, particularly in factors related to high yield titer. Further study is needed to evaluate behavioral risk factors associated with increased Widal titer and the cut-off level for Widal titer in population in Langsa City.

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