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RESEARCH ARTICLE
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Diagnostic Validity of Urinary Mucosal Anti – Salmonella typhi OMP Antibodies as Compared to Systemic Responses in Typhoid Patients

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ABSTRACT

Outer membrane protein [OMP] 55kDa and 62kDa antigen was identified from S. typhi and was loaded onto tanned Sheep's RBCs and be the diagnostic antigen for typhoid. Urine and blood samples were collected from 60 typhoid patients and 30 control individuals. Urinary Mucosal globulins [UMG] were separated from urine samples by PEG 6000 6%. Biuret color reaction was used to identify urinary mucosal globulins UMG. Sera were saved from patients and control blood samples. OMP antigen was titrated with UMG and sera using Passive hem-agglutination [PHA]as compared to S. typhi "O" and "H" titrated with UMG and sera by standard agglutination [AG], the classical Widal test. Titers were affixed for both of the tests. Sensitivity and specificity indices were calculated for AG and PHA. OMP – PHA was found to be more specific and sensitive than Widal. The majority of typhoid patients were showing anti -OMP, anti- O and anti- H antibodies both and mucosal and systemic compartment. OMP – PHA and Widal be of use as a battery for serodiagnosis and herd immunity of typhoid patients. The novelty of this study was the suggestion that urinary mucosal immunity can be used for typhoid diagnosis when patients systemic immune responses were negative.

Keywords: *Anti – Salmonella typhi, Antibodies, Typhoid Patients*

INTRODUCTION

Enteric – Typhoid fever ETF holds the position of endemicity in the Afro-Asian and the Indian Subcontinents countries (1). ETF seems to be a public health problem in the aforementioned countries in the; past, present and future . Cases are reported tell 2022 (2). Traditionally, blood serology, blood, bone marrow, fecal and urine cultures were the diagnostic laboratory aids for diagnosis of ETF (3,4). Urine as a tool in diagnosis of human infectious diseases have been reviewed by Al-Tai et al (5). ETF may release S. typhi in urine (6).

Salmonella typhi antigen has been demonstrated in ETF patient urine (7,8,9). Late in nineteen fifties, urine antibody had been detected in typhoid patients (10). Salmonella typhi autoantibody was demonstrated in urine of Lupus patients, workers attributed Salmonella autoantibody in pathogenesis of Lupus (11). Early in in Nine-teens of the last century the outer membrane protein OMP of S. typhi had been first tempted for diagnosis of typhoid fever (12). Then workers allover the world have been proved that OMP was ; immunogen& vaccine adjuvant(13), vaccine candidate(14).

All what have been mentioned was regarding. Antibody responses in blood sera of the patients tested by agglutination, lateral flow immune-chromatography and indirect ELISA (3,4,15). Neither urine mucosal antibody response nor OMP has ever been tried for diagnosis of ETF in urinary compartment as compared to systemic compartment. The present communication tempted to match urinary mucosal anti OMP Abs as compared to systemic anti OMP for typhoid patients.

MATERIALS AND METHODS

S. typhi

Five proven biochemically, serologically and molecular biologic identification were recovered from clinically and immunologically proven typhoid patients.

Outer membrane protein

S. typhi S5 isolate was used to separate, purify and identify outer membrane protein in accordance with (16). The OMP was loaded onto tanned Sheep's Red Cells and be the OMP diagnostic antigen (17).

Patients

Based on exclusion and inclusion criteria clinical review of patients were made by consultants medical internist of the local hospital at Najaf government at the period from November 2021 to June 2022. Sixty patients and thirty normal control subjects were the test and study groups.

Sampling

Five ml urine and blood samples from each of the patients and controls were collected. Sera were collected. Sera were saved from clotted centrifuged blood (17). Urinary mucosal globulins were separated by PEG 6000 6% (18). The separated pellets were checked by Biuret test and found to be of a protein identity after reconstitution with formal saline solution.

Qualitative Immunoassay

Typhoid rapid lateral flow immunochromatography test were made on sera of patients and controls (In accordance with manufactural recommendation).

Quantitative Immunoassay

S. typhi "OMP", "O" and "H" antigens as were titrated with urinary mucosal globulins solution and sera using standard agglutination and passive hem-agglutination tests(17,19).

RESULTS

Demography

The male - female ratio was 1.7:1. The age ranges of the patients were from 7 to 64 years and the cases were clustered around 20 to 44 years. Controls were of matching age groups.

Qualitative Immune Assessment

The 60 patients were showing S. typhi IgM 30:60 (50%), S. typhi IgM-IgG 23:60 (38.33%) and IgG 7:60 (11.66%), Figure (1).

(A) (B) (C) (D)

FIGURE 1: Lateral Flow Immunochromatography test (rapid test) for typhoid patients. This figure shows all results of typhoid diagnosis in latera flow chromatography test, where:(A) IgM positive, (B) IgG positive, (C) IgM & IgG positive and (D) Negative result.

Quantitative Immune Assessment

Urinary Mucosal Response

The urinary mucosal S. typhi specific anti – OMP hemagglutinins titer were ranging from 8 – 256 as compared to control as 2 – 4, while S. typhi anti – O mucosal Abs titers were ranging from 8 – 512 and S. typhi anti – H mucosal Abs titers were ranging from 8 – 512 as compared to normal of 2 – 4 titers for anti – O and anti – H . Table -1.

TABLE 1: Urine Immunoassay and biometry of typhoid patients

Features	Anti – OMP	Anti – O	Anti – H
Statistics			
Minimum	2	2	2
Mean	49.233	52.066	40.333
Median	16	12	16
Maximum	256	512	512
Range	254	510	510
Quality control			
Sensitivity Index	71.66%	68.33%	80%
Specificity Index	96.66%	90%	93.33%
Herd Immune response			
Low responders			
Moderate responders	8 – 16	8 – 16	8 – 16
High responders	32 – 64	32 – 64	32 – 64
	128 – 256	128 – 512	128 – 512
Herd Plot Nature	Skewed plot	Skewed plot	Skewed plot

Serum Antibody Response

The systemic S. typhi OMP hemagglutinin titers was 320 – 2560. While for anti – O and anti – H were ranging from 160 – 2560 in patients as compared to normal anti – OMP, anti – O and anti – H were 10 – 40, 10 – 40, Table -2.

TABLE 2 : Serum Immunoassay and biometery of typhoid patients.

Features	Anti – OMP	Anti – O	Anti – H
Statistics			
Minimum	80	10	80
Mean	1484	1404.5	1438.666
Median	1280	1280	1280
Maximum	2560	2560	2560
Range	2480	2550	2480
Quality control			
Sensetvity Index	98.33%	88.33%	98.33%
Specificity Index	96.66%	76.66%	83.33%
Herd Immune response			
Low responders			
Moderate responders	80	10 – 80	80
High responders	320 – 640	160 – 640	160 – 640
	1280 - 2560	1280 - 2560	1280 - 2560
Herd Plot Nature	Gaussian plot	Gaussian plot	Gaussian plot

Comparative View

The systemic anti – OMP titer were mostly higher than mucosal antibody titers likewise. The systemic anti – O, anti – H were higher than mucosal Abs titers in patients. The patient's titer for anti – OMP, anti – O and anti – H were higher

than that of controls. The anti – OMP were clustered around 640 – 2560 in serum corresponds to 128 – 256 in patient's urine, while they were clustered around 1280 for anti – O in serum at 64 – 256 in urine of the patients. The mean, median, minimum and maximum and ranges for titers were depicted, Table-1 and Table-2. The Systemic to mucosal mean rate for anti – OMP in serum to urine, anti – O serum to urine and anti – H serum to urine were 720/292 , 2.5/1, 840/19.6, 4.3:1 , 840 / 203.2 and 4.1/1.The index of sensitivity and the index of specificity for OMP – PHA were 68.88% and 96% , while for Widal were 68.88% and 76.98% for O and 93% - 98%, 83% - 94% for H respectively. The recommended battery tests for typhoid were typhoid, OMP – PHA and Widal for systemic, while for mucosal was OMP – PHA and Widal.

Typhoid Immunity and typhoid herd immunity

Typhoid patients have shown two immune response patterns as; Systemic-Mucosal, and systemic only for OMP-PHA. While for Widal test, the patterns were as; Systemic-Mucosal, mucosal only and systemic only, as it is shown in ,Table -3

TABLE 3: Typhoid Immune response Patterns

Assay	Response patterns	Observed incidence
OMP-PHA	Systemic-mucosal Systemic	57:60 (95%) 3:60 (5%)
Widal test	Systemic-mucosal Mucosal Systemic	54:60(90%) 5:60(8.33%) 1:60(1.66%)

The anti-OMP, anti-O and anti-H antibodies were satisfactory for mapping herd immune plots of the test typhoid population. They were either of skewed or Gaussian distribution plots that are illustrated in Figures (2), (3), (4), (5), (6) and (7).

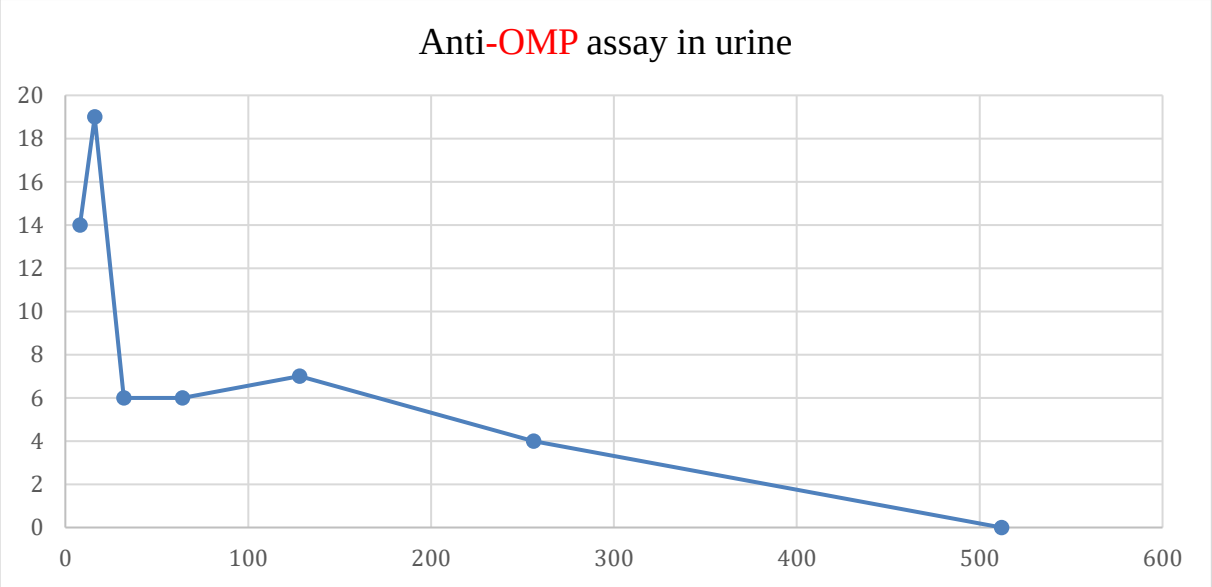

FIGURE 2: Anti-OMP assay in urine. This figure illustrates positive skewed plots that explains herd immune responders which include low, moderate and high responders for anti- OMP antibodies in urine of typhoid patients.

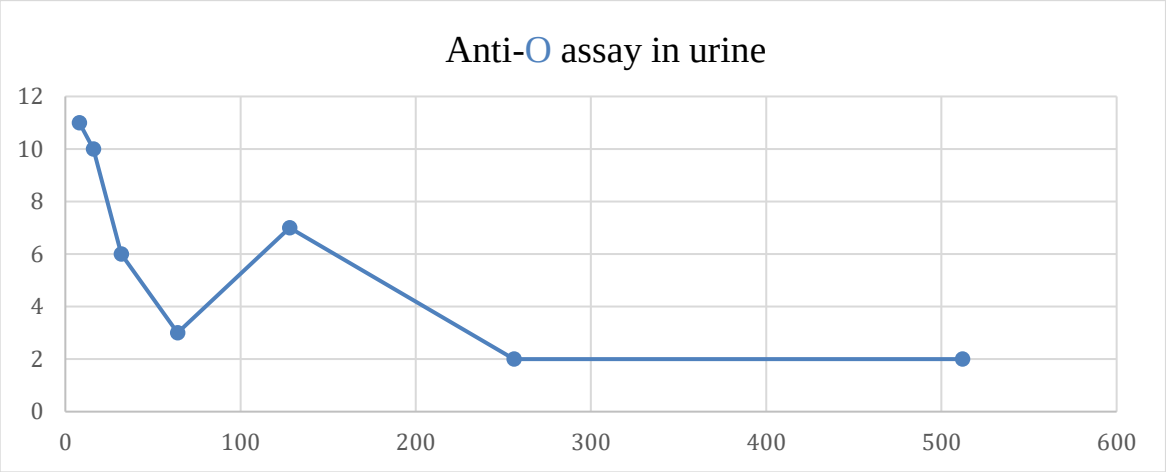

FIGURE 3: Anti-O assay in urine. This figure illustrates positive skewed plots that explains herd immune responders which include low, moderate and high responders for anti-O antibodies in urine of typhoid patients.

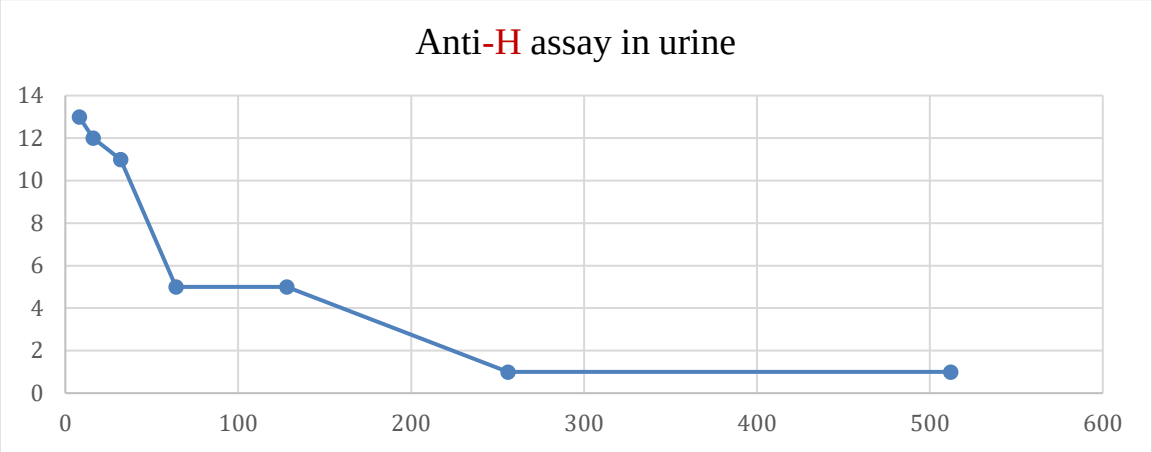

FIGURE 4: Anti-H assay in urine. This figure illustrates positive skewed plots that explains herd immune responders which include low, moderate and high responders for anti-H antibodies in urine of typhoid patients.

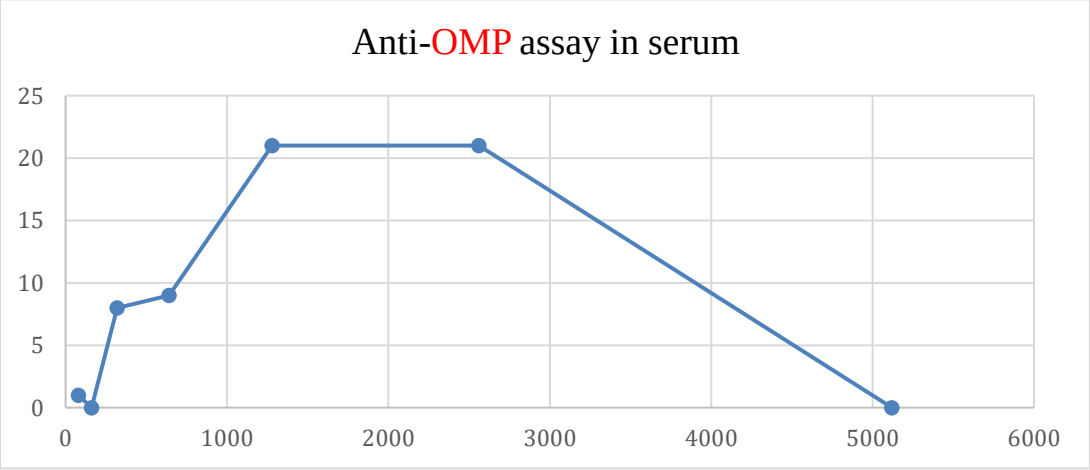

FIGURE 5: Anti-OMP assay in serum. This figure illustrates Gaussian distribution plot that explains herd immune responders which include low, moderate and high responders for anti- OMP antibodies in sera of typhoid patients.

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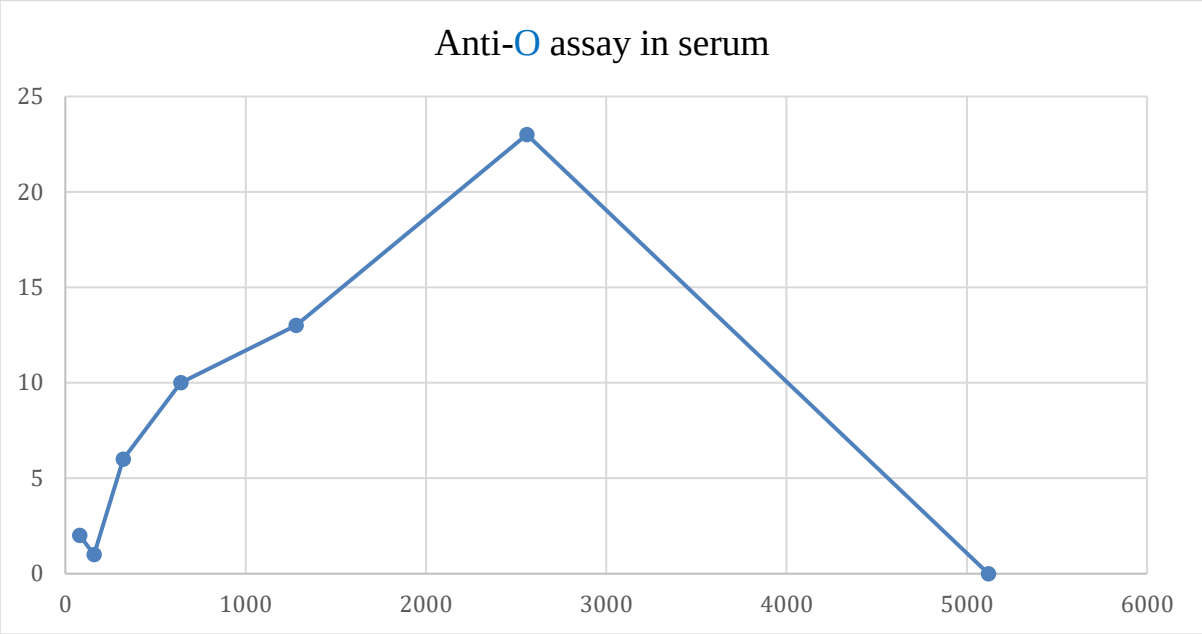

FIGURE 6: Anti-O assay in serum. This figure illustrates Gaussian distribution plot that explains herd immune responders which include low, moderate and high responders for anti-O antibodies in sera of typhoid patients.

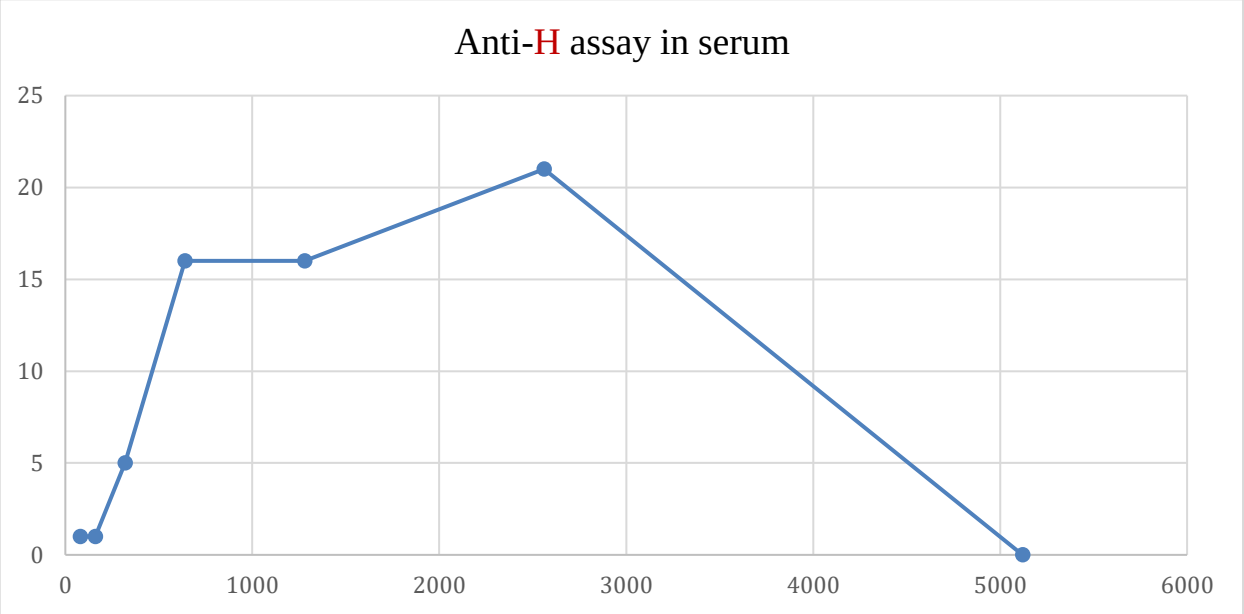

FIGURE 7: Anti-H assay in serum. This figure illustrates Gaussian distribution plot that explains herd immune responders which include low, moderate and high responders for anti-O antibodies in sera of typhoid patients.

DISCUSSION

The present study, table 1,2 and 3, tried to pin-point the importance of urine antibodies as an adjunct to serum antibodies for the serodiagnosis of typhoid. In which Salmonella typhi outer membrane protein OMP 55kDa and 62kDa was tempted for serodiagnosis of typhoid through passive hemagglutination. As compared to Widal test. The sensitivity and specificity indices for PHA and Widal done on patient's serum and UMG were comparable to that have been documented by other workers(20-29), Table -4.

TABLE 4 : Quality control of typhoid immunoassays.

Test	Index of specificity	Index of sensitivity	Geographic area	Reference
Widal	69.26%	84.23%	Indonesia	(20)
	47.6%	88.89%	Nairobi	(21)
	55.49%	36.70%	Pakistan	(22)
	78.0%	30%	India	(23)
	76.0%	100%	Uganda	(24)
	81.25%	90%	South India	(25)
	53.0%	80%	Nepal	(26)
	89.5%	86.7%	Egypt	(27)
	61.7%	83.3%	Iraq / Tikrit	(28)
	41.3%	92.45%	Iraq / Tikrit	(29)
Dot EIA	90.6%	93.3%	Egypt	(27)
ELISA IgM	54%	95%	India	(26)
IgG	95%	96%	India	(26)
Typhi dot	81.6%	75.0%	India	(25)
Tubextp	63.34%	42.65%	Pakistan	(22)
	69.64%	84.27%	Indonesia	(20)
	95.0%	97.7%	Nairobi	(21)

Parallels to the use anti OMP, anti O and anti H in urinary mucosal antibodies in typhoid patients. Urinary mucosal antibodies had been used for diagnosis of persistent pyuria (18) and gut mucosal antibodies in typhoid patients ((30). The anti OMP antibodies in typhoid patients may be of help preventing attachment of Salmonella to urinary bladder mucosa (4) or might form antibody coated bacteria (31). The mean values of serum anti OMP, anti O and anti H that of mucosal responses were found of importance in pathogenesis and immunity to typhoid disease (30,32,33,34). The principle of herd immunity were found applicable in typhoid (35,36). Hence, four achievements were established in this study Table 1,2 and 3. First, OMP 55kDa and 62kDa(15) were tempted as an antigenic bioreagents for serodiagnosis of typhoid. Second, the use of patients humoral immune conversion from base-line titers to clinical indicative titre through the use of anti OMP, anti O and anti H antibodies in typhoid diagnosis ,third the use of Salmonella typhi antibodies in tracing herd immunity in typhoid patients (35,36) and fourth the suggestion of urinary mucosal immune responses can be of use for diagnosis of typhoid patients where no evident positive systemic responses.

CONCLUSION

Specific anti OMP antibodies where matched into two immune response patterns as systemic - mucosal and systemic only. While S. typhi anti O and anti H antibody responses patterns where matched in three patterns ; systemic – mucosal, mucosal and systemic alone. Index of sensitivity and index of specificity anti OMP, anti O, anti H were within the limits reported in Afro-Asian and the Indian Subcontinents countries. These antibodies were found of use in mapping humoral herd immunity in typhoid patients herd. The plots were of skewed plots type. Urinary mucosal immune responses can be of use for diagnosis of some typhoid patients were no evident systemic response.

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