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MALARIA AND TYPHOID FEVER: POSITIVE WIDAL TEST IN MALARIA PATIENTS REPORTED AT TERTIARY CARE HOSPITAL

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Background:
Illness due to Malaria and Typhoid co-infection is a most essential community wellbeing issue in most of the rising nations. Many of the co-infections treated are established on techniques of diagnosis afflicted with expectations that may exaggerate the circumstances.
Therefore the objective of current study was to analyze the level of co-infection by using the Widal test for diagnosis of typhoid fever in cases of Malaria at Nawabshah, Pakistan.
Methodology:
An aggregate of 390 subjects who were positive for malaria were reanalyzed for typhoid fever. Blood samples were collected from all subjects positive for malaria and were sent to laboratory for initial evaluation of laboratory diagnosis of Typhoid fever.
Results:
Out of 390 subjects positive for malaria parasite, there were 52 subjects positive for typhoid fever by the Widal test. The ratio of malaria and typhoid was equal to 7.5:1. The level of co-infection was considerably extraordinary when symptoms were more at presentation. Typhoid was screened by Widal Test. Analysis showed an association among malaria parasite and the level of antibody titers of Salmonella in subjects suffering from malaria.
Conclusion:
Co-infection of malaria and typhoid is frequent in endemics of Nawabshah Pakistan.
Key words: *Widal test, MP ICT Antigen, Malaria, typhoid fever, co-infection.*

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INTRODUCTION:

Universally 216 million cases of malaria were reported in 2016, in comparison to 237 million reported cases in 2010 and 211 in 2015. The greatest portion (90%) of reported cases was from WHO African region, 7% from WHO South East Asian region and 2% from WHO eastern Mediterranean region. From 91 countries (in 2016) that reported malaria cases, 80% of the universal malaria burden was from 15 countries including all in Sub-Saharan Africa and India. It is assessed that the frequency of malaria had decreased universally up to 18% i.e. decreased from 76/1000 to 63/1000 population at risk. There was 48%, 22% and 20% reduction in malaria cases in WHO regions South East Asia, America and Africa respectively.[1]

Universally in 2013, approximately 198 million cases and 0.584 million deaths were reported due to malaria and a decrease in incidence (30%) and mortality (47%) rates ever since 2000 were also noted [2][3].Malaria is well thought-out as an ailment of poverty and a well-known community health issue; it has vast medical, social and economic effects on community [4]. About 60% of our population lives in the malaria endemic areas [5][6]. Malaria is endemic in Pakistan since many decades; a recent surge was seen in malaria affecting more than 60 million populations from 60 districts that was attributed to floods [6]. In Pakistan 0.5 million cases of malaria are reported each year with about fifty thousand deaths per year [7]. It is estimated that about 37% of the all malaria cases are reported from the regions near the borders of Afghanistan and Iran [8].

On the other hands, typhoid fever which is also simply called as 'typhoid' is a bacterial fever caused by Salmonella typhi equally called Salmonella enterica serotype Typhi [9][10].

The contamination of food and/or water with Salmonella enteric serotype typhi from faeces of an infected subject is the most common reason of acquiring the typhoid infection, humans are the only infected after the consumption of the contaminated food/water [11]. Poor sanitation and poor hygiene are the risk for acquiring typhoid infection [10].

In the year 2000 and 2010, an estimated 21.7 million and 13.5 million typhoid fever illnesses were recorded. Between years 2000 and 2013, it resulted in estimated 217,000 and 161,000 deaths respectively [12][13].The highest load of disease is seen in newborns, youngsters, and adolescents in Southeast and south-central Asia [14].

Owing to the geographical overlap of both infections, co-infections are very common. Though, the specific prevalence of the concomitant malaria and typhoid fever in most of the geographic regions is unclear, as they had mutually same community conditions which are essential to their spread; in regions where both ailments are common; community is at high risk of having co- infections simultaneously or an acute or chronic [15].

Malaria is a substantial problem of the underprivileged and most susceptible populations of district Shaheed Benazirabad and its peripheries in Sindh Pakistan.

This paper represents a study performed to conclude the frequency of malaria, typhoid and their confection in District Shaheed Benazirabad and its peripheries.

MATERIALS & METHODS:

Objective: The study was aimed at determining the frequency of infection by malaria, and co infection by these in the subjects with fever.

Operational Definitions:

Malaria:

Malaria is an infectious disease of parasitic protozoans (a single celled micro-organism) related to Plasmodium causing diseases in human beings and also other animals. All the species of plasmodium causes diseases. P. falciparum causes severe diseases. On the other hand P. vivax, ovale and malariae commonly result in mild disease. P. knowlasi may rarely cause disease in mankind and /or antigen based tests (ICT) are used for the malaria diagnosis [16][17].

Typhoid fever:

Typhoid also known as the enteric fever is a systemic febrile illness and is caused by certain serotypes of salmonella (Salmonella enterica serotype typhi (S. typhi) and Salmonella enterica serotype paratyphi). S. typhi is the most common serotype of salmonella that causes typhoid fever [18][19].

DESIGN: This study was cross sectional.

Area of Study: Nawabshah is situated along the rainforest strap and located between Latitude 6.030N and Longitude 6.90E. Peoples Medical University Hospital is situated in Nawabshah. Nawabshah is densely populated with mix population living in urban and rural areas. Occupation of community includes agriculturalists, merchants, civil servants and limited number of community health employees. Cleanliness is very poor with unsatisfactory drainage structure. For this study district Shaheed Benazirabad and its peripheries were preferred due to reasons of a

large number of subjects attending the tertiary care hospital.

Sampling: In current study 985subjects were involved, from them 390 were positive for malaria. All subjects with positive malaria test were analyzed for the presence of typhoid co-infection by typhidot test. Sample size with 95% confidence level and 5% margin of error from total population of about 1.6 million with distribution response rate of 50% were calculated by using Rao Software.

Duration: The current study was carried out between January 2017 and August 2018.

Inclusion and exclusion criterion

All patients of either gender with fever and clinical history of malaria, positive for malaria parasite antigen and after that Widal positive were included and patients not willing for taking part in study, known cases of blood disorders, HBsAg, sickle cell disease were excluded from study.

Ethical Considerations

Research and Ethics Committee of the People's Medical University Nawabshah approved to conduct the study, while permission was obtained from the patients and next of kin. Consent was sought and obtained from participants or their relations and only those who gave their consents were enrolled into this study. Information or results obtained from the study was treated with utmost confidentiality and used for the purpose of the research only.

Data collection:

Different variables such as sex, age, address, presence of malaria parasite and type of plasmodium of malaria parasite of patient were obtained subsequent to brief consultation. Clinical examination of subjects was carried out for malarial, typhoid and malaria with typhoid diagnosis. Sampling of subjects was related to suspicious of malaria and typhoid.

For Widal test venous blood was collected in cleaned dry test tubes and allowed to clot for serum separation. Standard Salmonella antigen suspension in equal volume for O and H antigens were mixed with serum.

A fourfold or more increase above reference line of antibody titer (1:160) was regarded as significant.

Statistical analysis: For analysis of the collected data SPSS (statistical package for sciences) was used (version 20.0) Chi-square (χ^2) determined the relations among malaria ICT antigen and Widal /Typhidot also the Bivarate Correlation and chi-square (χ^2), were also utilized for the data analysis.

RESULTS:

This study was carried out with certain limitations but lot of helpful in the management of illness. 985 subjects presenting with fever were screened by MP ICT antigen for malaria and by Widal test for typhoid fever.

Out of 985 subjects, 390 were positive for malaria and 52 subjects were positive for typhoid (from all 390 malaria positive subjects).

The ratio of malaria and typhoid was **7.5:1.** In subjects with more symptoms at presentation the rate of co-infection was significantly great. Figure 1.

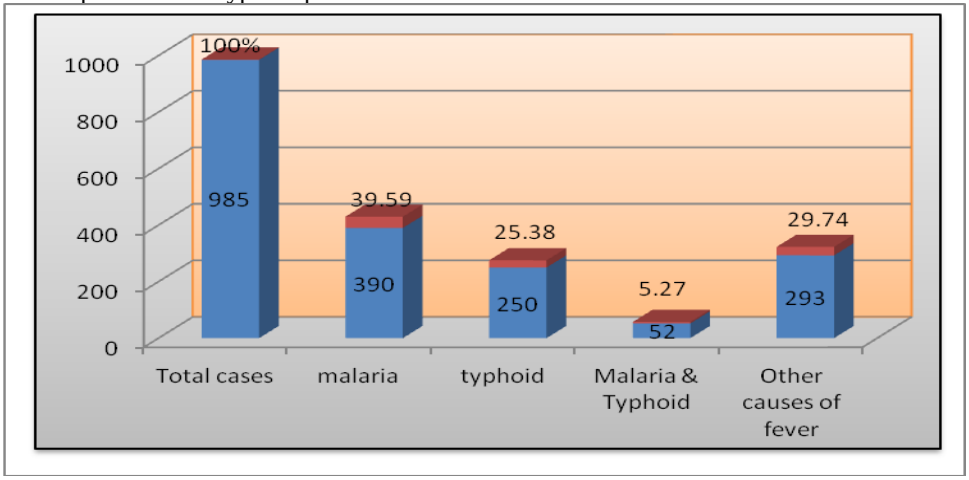


Table 1. Age statistics

The mean age of subjects participating in current study was 38.63 with Std. Deviation ±13.68 years, the Minimum age 18.00 and Maximum age was 77.00 years respectively.

Table 1. Age Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Age(Years)	390	18.00	77.00	38.6308	13.68230
Valid N (listwise)					

DEMOGRAPHIC VARIABLES:

Different variables of study with their sub titles, frequency, percentage, valid percentage, cumulative percentage, chi=square, df and Asymp. Sig.were assessed in table 2.

Out of **390** subjects, males 53.6 % and 46.4 % were females (p <0.156). There were 93.3 % married and 6.7 % unmarried (p <0.000). 56.9 % subjects were from young age group while 33.6 % and 9.5 % were from middle and old age group respectively. By occupation, House wives were 47.9%, manual workers 30.3%and office workers 21.8%(p <0.000).

Majority of subjects (72.1%)belonged to rural community and 27.9% were from urban community(p <0.000).

Regarding socio-economical status 91.5 % subjects were from lower, 5.6% middle and 2.9% were from upper class respectively (p <0.000).

While regarding addiction status 58.5% subjects were not addicted while 39.5% were addicted to different substances in their life. p value <0.001

The education profile was also assessed in current study showing the different status of education levels here There were 15.6% subjects uneducated; while 42.6%were primary, 20.3% middle to matriculation, 12.3% intermediate and 9.2% graduate respectively. p value <0.000

The type of plasmodium shown that there were 52.1% cases were plasmodium falciparum while 34.9% were plasmodium vivax, 1.0% plasmodium malaria and 11.78% were mix infection of plasmodium falcipaurum and vivax.p value <0.000

Widal test was negative in 86.7% and positive in 13.3% patients.

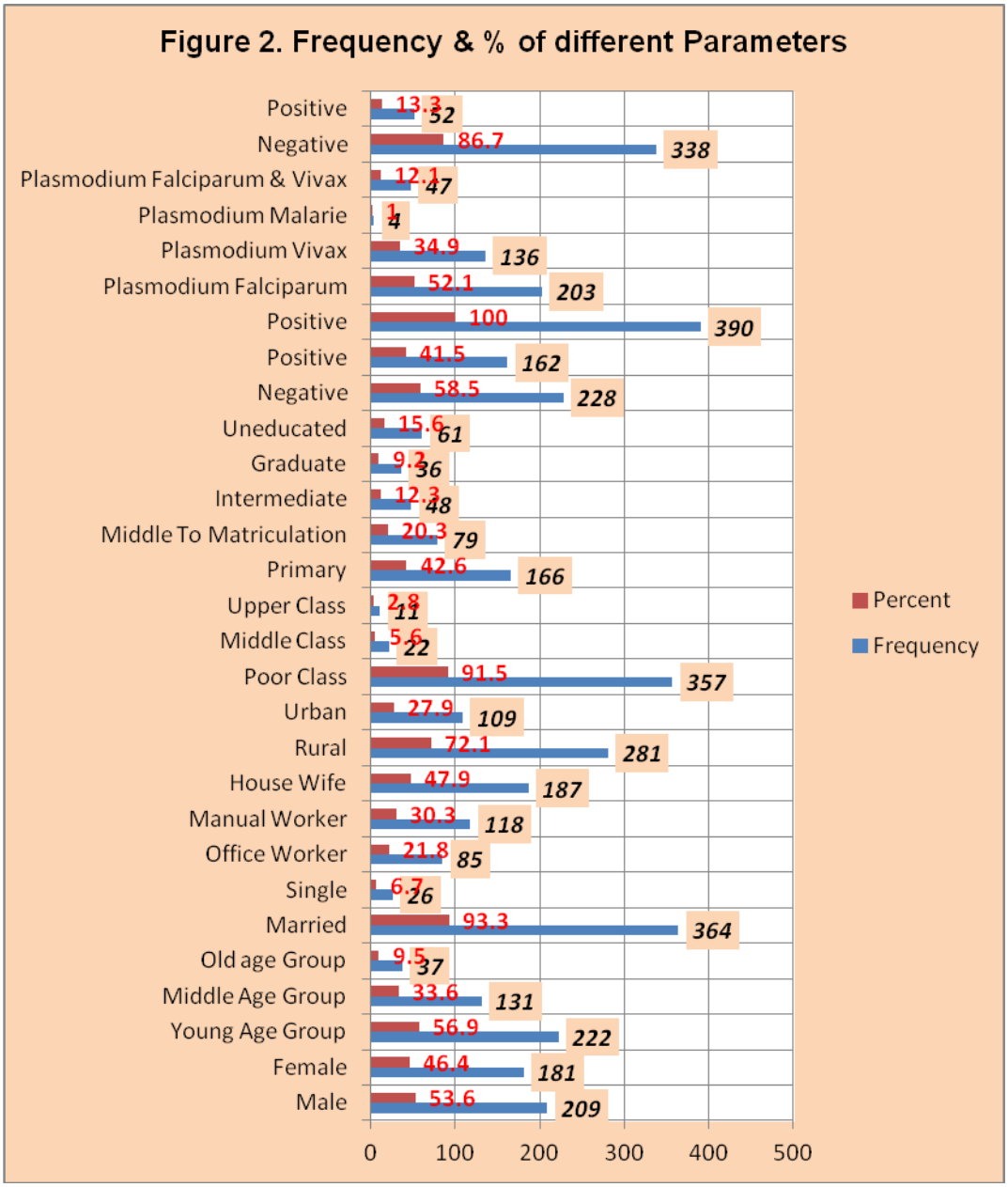


Table 2. Non parametric chi square test

The non parametric chi square test had shown that the asymp. Sig was <0.001 in all variables except gender sig. 0.156 and addiction sig. 0.001. These results were significant statistically.

Table 2. Non parametric chi-square test of variables. N=390											
	Age In Years	**Age_ Group**	**Gender**	**Ms**	**Occupation**	**Address**	**S_E**	**Education**	**Addiction**	**Type Of Plasmodium**	**Widal Test**
Chi-Square	177.969	131.646	2.010	292.933	41.677	83.077	595.031	131.923	11.169	245.179	209.733
df	53	2	1	1	2	1	2	4	1	3	1
Asymp. Sig.	.000	.000	.156	.000	.000	.000	.000	.000	.001	.000	.000

Table 3. Malaria Parasite Antigen and Widal test Gender Crosstabulation

Out of 209 male cases of malaria, Widal test was positive in 16.3% male patients and negative in 83.7% patients. Out of 181 female cases of malaria, Widal test was positive in 9.9% female patients and negative in 91.1% patients

Table 3. malaria parasite antigen * widal test * gender Crosstabulation						
				widal test		
Gender				negative	positive	Total
Male	Malaria Parasite Antigen	Positive	Count	175	34	209
			% of Total	83.7%	16.3%	100.0%
	Total		Count	175	34	209
			% of Total	83.7%	16.3%	100.0%
Female	Malaria Parasite Antigen	Positive	Count	163	18	181
			% of Total	90.1%	9.9%	100.0%
	Total		Count	163	18	181
			% of Total	90.1%	9.9%	100.0%

Table 4. Gender Type of Plasmodium Widal test Cross tabulation

There were total 390 cases of malaria that were included in present research, out of them Widal negative males 51.8% were with plasmodium falciparum, plasmodium vivax, Plasmodium Malarie, Plasmodium falciparum with Vivax. Widal negative females were 48.2% with plasmodium falciparum, plasmodium vivax, Plasmodium Malarie, Plasmodium falciparum with Vivax. Widal positive males 65.4% were with plasmodium falciparum, plasmodium vivax, Plasmodium Malarie, Plasmodium falciparum with Vivax. Widal positive females were 34.6% with plasmodium falciparum, plasmodium vivax, Plasmodium Malarie, Plasmodium falciparum with Vivax.

For assessment of negative widal test with type of plasmodium and gender category different chi-square tests were performed with Pearson chi square 1.089, df 3, Asymp. sig.(2-sided).780.Likelyhood ratio 1.135with df 3, Asymp. sig.(2-sided).769. Linear by linear association .111 with df 1, Asymp. sig.(2-sided) .739.For assessment of positive widal test with type of plasmodium and gender category different chi-square tests were performed with Pearson chi square .390, df 2, Asymp. sig.(2-sided).823.Likelyhood ratio .407 with df 2, Asymp. sig.(2-sided).816. Linear by linear association .365 with df 1, Asymp. sig.(2-sided) .545.

For negative Widal test Symmetric Measures were also analysed for Interval by interval pearsons R value -.018 with Approx. Sig .739, Ordinal by ordinal Spearman correlation value -.004, Approx. Sig. .938, For positive Widal test Symmetric Measures were also analysed for Interval by interval pearsons R value -.085 with Approx. Sig .551, Ordinal by ordinal Spearman correlation value -.067, Approx. Sig. .638, all were assessed statistically and different values were shown in Table.

Table 4. Gender, Type of Plasmodium & Widal test Cross tabulation

Widal Test				Type Of Plasmodium				Total
				Plasmodium Falciparum	Plasmodium Vivax	Plasmodium Malarie	Plasmodium Falciparum & Vivax	
Negative	Gender	Male	Count	90	61	3	21	175
			% of Total	26.6%	18.0%	.9%	6.2%	51.8%
		Female	Count	83	61	1	18	163
			% of Total	24.6%	18.0%	.3%	5.3%	48.2%
	Total		Count	173	122	4	39	338
			% of Total	51.2%	36.1%	1.2%	11.5%	100.0%
Positive	Gender	Male	Count	19	9		6	34
			% of Total	36.5%	17.3%		11.5%	65.4%
		Female	Count	11	5		2	18
			% of Total	21.2%	9.6%		3.8%	34.6%
	Total		Count	30	14		8	52
			% of Total	57.7%	26.9%		15.4%	100.0%

Chi-Square Tests

Widal Test		Value	df	Asymp. Sig. (2-sided)
Negative	Pearson Chi-Square	1.089[a]	3	.780
	Likelihood Ratio	1.135	3	.769
	Linear-by-Linear Association	.111	1	.739
	N of Valid Cases	338		
Positive	Pearson Chi-Square	.390[b]	2	.823
	Likelihood Ratio	.407	2	.816
	Linear-by-Linear Association	.365	1	.545
	N of Valid Cases	52		

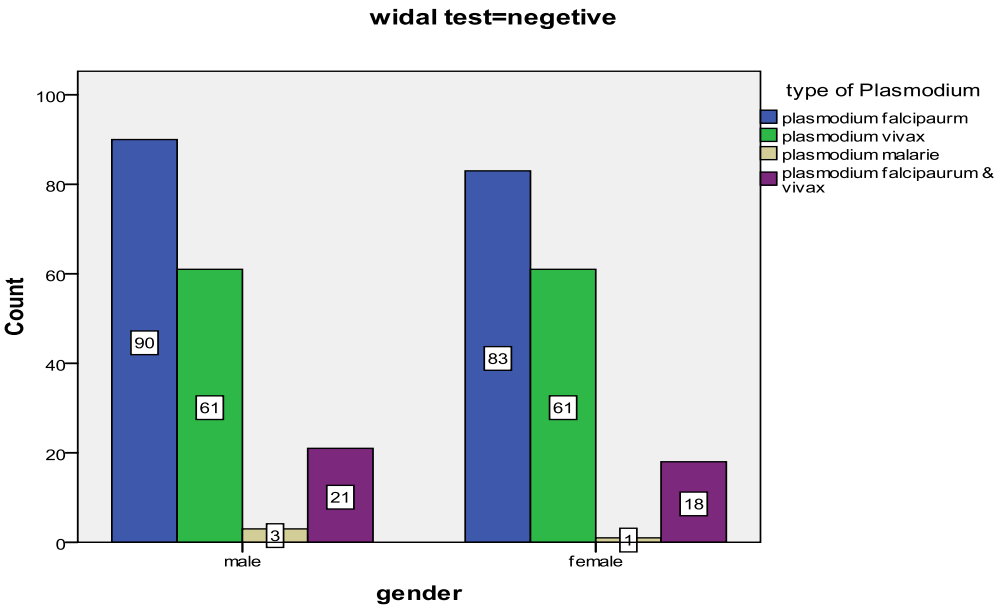
Symmetric Measures

Widal Test			Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Negative	Interval by Interval	Pearson's R	-.018	.054	-.333	.739[c]
	Ordinal by Ordinal	Spearman Correlation	-.004	.054	-.078	.938[c]
	N of Valid Cases		338			
Positive	Interval by Interval	Pearson's R	-.085	.130	-.601	.551[c]
	Ordinal by Ordinal	Spearman Correlation	-.067	.135	-.474	.638[c]
	N of Valid Cases		52			

Negative Widal test

Widal test was negative in 90 male patients with plasmodium falciparum, 61 cases plasmodium vivax, 3 cases of plasmodium malaria and 21 cases of plasmodium falciparum and vivax.

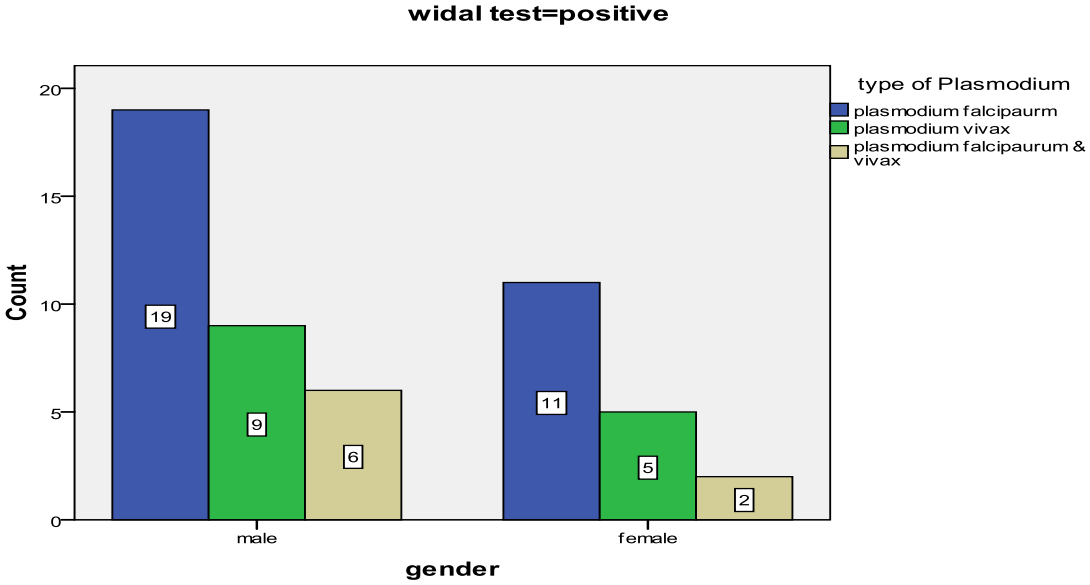
Widal test was negative in 83 female patients with plasmodium falciparum, 61 cases plasmodium vivax, 1 cases of plasmodium malaria and 18 cases of plasmodium falciparum and vivax. Figure 3.



POSITIVE WIDAL TEST:

Widal test was positive in 19 male patients with plasmodium falciparum, 9 cases plasmodium vivax, and 6 cases of plasmodium falciparum and vivax.

Widal test was positive in 11 female patients with plasmodium falciparum, 5 cases plasmodium vivax, and 2 cases of plasmodium falciparum and vivax. Figure 4.



CORRELATION:

The correlation of Widal test and malaria with their different plasmodium types and demographic variables of study were assessed by bivarate analysis. There was strong correlation among Widal test with type of plasmodium, age in years with age group and addiction occupation, gender with marital status and occupation, addiction with age in years and age group, type of plasmodium with Widal test, educational status. The other parameters of study were not showing significant correlation with Widal test as shown in table 5.

Table 5. Correlations

		Widal Test	Age In Years	Age_ Group	Gender	Ms	Occupation	Address	S_E	Education	Addiction	Type Of Plasmodium
Widal Test	Pearson Correlation	1	-.037	-.049	-.093	.016	-.139**	-.051	-.036	-.070	-.040	.007
	Sig. (2-tailed)		.468	.331	.067	.751	.006	.315	.483	.166	.433	.889
Age In Years	Pearson Correlation	-.037	1	.891**	-.059	-.108*	-.009	.028	.030	.040	.493**	-.067
	Sig. (2-tailed)	.468		.000	.247	.033	.862	.578	.561	.427	.000	.185
Age_ Group	Pearson Correlation	-.049	.891**	1	-.063	-.088	.002	-.002	.038	.029	.525**	-.026
	Sig. (2-tailed)	.331	.000		.213	.083	.971	.974	.456	.572	.000	.607
Gender	Pearson Correlation	-.093	-.059	-.063	1	.164**	-.203**	-.078	.021	-.064	-.065	-.028
	Sig. (2-tailed)	.067	.247	.213		.001	.000	.124	.686	.210	.204	.582
Ms	Pearson Correlation	.016	-.108*	-.088	.164**	1	-.153**	-.070	-.076	-.014	-.017	-.060
	Sig. (2-tailed)	.751	.033	.083	.001		.002	.171	.133	.788	.743	.240
Occupation	Pearson Correlation	-.139**	-.009	.002	-.203**	-.153**	1	-.062	-.020	.049	.004	-.038
	Sig. (2-tailed)	.006	.862	.971	.000	.002		.224	.687	.335	.935	.458
Address	Pearson Correlation	-.051	.028	-.002	-.078	-.070	-.062	1	-.056	.110*	.005	.002
	Sig. (2-tailed)	.315	.578	.974	.124	.171	.224		.268	.030	.929	.962
S_E	Pearson Correlation	-.036	.030	.038	.021	-.076	-.020	-.056	1	.048	.023	.036
	Sig. (2-tailed)	.483	.561	.456	.686	.133	.687	.268		.347	.655	.478
Education	Pearson Correlation	-.070	.040	.029	-.064	-.014	.049	.110*	.048	1	.058	.141**
	Sig. (2-tailed)	.166	.427	.572	.210	.788	.335	.030	.347		.255	.005
Addiction	Pearson Correlation	-.040	.493**	.525**	-.065	-.017	.004	.005	.023	.058	1	.010
	Sig. (2-tailed)	.433	.000	.000	.204	.743	.935	.929	.655	.255		.845
Type Of Plasmodium	Pearson Correlation	.007	-.067	-.026	-.028	-.060	-.038	.002	.036	.141**	.010	1
	Sig. (2-tailed)	.889	.185	.607	.582	.240	.458	.962	.478	.005	.845	

Paired Samples Test , Paired Samples Statistics & Paired Samples Correlations type of plasmodium and widal test.

The paired sample test with 95% confidence interval along was analyzed and found that there was statistically significant relationship between type of plasmodium and Widal test sig. (2-tailed <0.001). Paired statistics and correlation has also shown that correlation was 0.007 and sig. was .889. Table 6.

Table 6. Paired Samples Test, Paired Samples Statistics & Paired Samples Correlations type of plasmodium and Widal test.

Paired Samples Test

Type Of Plasmodium - Widal Test	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
	.72821	1.31941	.06681	.59685	.85956	10.899	389	.000

Paired Samples Statistics

Type Of Plasmodium Widal Test	Mean	Std. Deviation	Std. Error Mean	N
	1.8615	1.27717	.06467	390
	1.1333	.34037	.01724	390

Paired Samples Correlations

Correlation	Sig.
.007	.889

DISCUSSION:

Commonly infectious ailments in tropical regions present with fever. Many of curable or avoidable pathogens that are recognized to result fever in subjects presenting with malaria-like symptoms, but have no malaria [20]. In tropical Africa malaria and typhoid fevers are major community health ailments and cause of mortality and morbidity [21][22].

Malaria and typhoid both are common in many nations of the globe due to environmental circumstances like warm moistly weather, decreased hygienic practices, poverty and lack of knowledge. Both of these ailments had been related with poverty and under development [21].

Malaria and typhoid both are the chief health problem of communities in tropics and subtropics. Subjects living in the endemic regions are at risk of adopting both infections simultaneously.

Current study was designed to determine the pervasiveness and related risk factors of malaria, typhoid, and co-infection amongst subjects with fever. Malaria a febrile disease that is caused by one or more plasmodium species is a common fatal ailment of the globe. Plasmodium species that infect humans are; plasmodium falciparum, vivax, ovale, malariae, and knowlesi. Around half of the globe inhabitants are at risk of infection by malaria. Sub Saharan Africa is the region from where most of the malaria cases and deaths are reported. About 216 million cases and 655,000 deaths were related to malaria in a report by W.H.O in 2011 [1]. Typhoid also known as the enteric fever is a systemic febrile illness and is caused by certain serotypes of salmonella (Salmonella enterica serotype typhi (S. typhi) and Salmonella enterica serotype paratyphi). S. typhi is the most common serotype of salmonella that causes typhoid fever [12][18][19][23]. In 2012 there were about 13.5 million cases of typhoid fever throughout world [12].The main causes of typhoid fever transmission in the developing countries are related with inadequate disposal of human excreta, ill-furnished latrines with poor facility of water, poor hand washing practices and use of untreated water [12][19]. Malaria and typhoid fever; one is caused by protozoa and other by gram negative bacilli and both are transmitted by separate mechanisms are the most important community health issues in tropics and subtropical regions [12][23][24][25]. Habitants of endemic regions are at risk of having both infections simultaneously [22][26]. The signs and symptoms of both diseases (Typhoid and Malaria) have a significant similarity [27][28][29][30][31]. So the overlapping of clinical features in both infections

usually leads to missed diagnosis and ultimately missed management in subjects with fever [28][32]. Thus reliable diagnostic methods are important for effective management of cases to reduce misuse and wastage of drugs [28][31][32][33].

So as far the occurrence of malaria, typhoid fever, their co-infection, and associated risk factors were not well studied in Nawabshah Pakistan. Current study was designed to determine the pervasiveness and related risk factors of malaria, typhoid, and co-infection amongst subjects with fever. Jamali AA et al analyzed cases of malaria with mix infections (p. falciparum and p vivax), there was dominant ratio of p. falciparum than p. vivax, while only 01% cases of plasmodium malaria were diagnosed and no case of p.ovale detected [34]. In another study by Shams et al males were in dominant ratio than females. Married were common than unmarried. There were prominent figure of subjects from young age group, than subjects from middle and old age groups respectively.[35]. In another study there was dominancy of infection by plasmodium vivax 60.25% in comparison to 39.75% patients who had infection by p. falciparum [36]. Study conducted by Jamali AA et al There was dominancy of plasmodium vivax in their study also. [37] Study had shown that 36.2% (127/350) have co-infection (both typhoid and malaria). Though the occurrence of malaria parasite was statically significant in association with gender ($p<0.05$), while typhoid pervasiveness was statically non-significant in association with gender ($p>0.05$). The co-morbid infection by malaria and typhoid was also statistically significant related to gender ($p<0.05$) [38]. In this study Out of 209 male cases of malaria, Widal test was positive in 16.3% male patients and negative in 83.7% patients. Out of 181 female cases of malaria, Widal test was positive in 9.9% female patients and negative in 91.1% patients. The findings of current research are in close limits and figures of past studies.

In another study co-infection of both malaria and typhoid was noted in 6.5% [39]. Co-infection with malaria and typhoid is usually seen in malaria endemic regions in the same way infection by typhoid had been related with malaria. Due to this fact many practitioners give combined treatment in probable case of typhoid or malaria. In a study 25 subjects of malaria were analyzed for salmonella antibodies and was seen that 23/25 (92%) had positive antibodies (titer $\geq$ 1/80). In 13/23 subjects stool samples were positive where as in 3/23 blood sample s were positive for typhoid. There was no association among malaria and typhoid infection, on the other hand there was statistically significant association ($p<0.01$) among different salmonella species (typhi, paratyphi B, paratyphi C). Also a significant relationship among salmonella in stool and blood ($p<0.05$) this suggests that existence of salmonella in blood is suggestive of its existence in stool also [40].

Recommendations

For proper diagnosis of malaria thick and thin film Geimsa stain and MP ICT antigen should be advised and for diagnosis of enteric fever blood culture and bone marrow culture should be encouraged when feasible.

CONCLUSION:

Infection by malaria and typhoid were common in the studied populace with increased rate of their co-infection. Female gender had higher ratio of co-infection in comparison to male gender. Struggles must be done in improving the living environments of the inhabitants of district Nawabshah and peoples must be clarified on the prevention as well as controlling methods of the both infections. As both ailments present with related signs and symptoms, so the management must be initiated after laboratory diagnosis. Personal hygienic conditions should be encouraged in the population.

Conflict of Interests

The authors declared no conflict of interests concerning the publication of paper.

Authors' Contribution

Shamasuddin Shaikh and Ghulam Mustafa Jamali enrolled the subjects, gathered and evaluated data, and composed the paper. Anwar Ali Jamali and Bhojomal Tanwani contributed in introduction and discussion, Aamir Shahzad Malik and Naveed Sattar Shaikh helped in data interpretations and statistics.

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