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Incidence of Typhoid Fever and Determination of Sialic Acid in Serum of the Patients at Nigerian Airforce Hospital Kano State, Nigeria

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Abstract

Typhoid fever remains a significant public health concern in many regions of the world. Over-dependence on clinical presentation and/or the widal agglutination test for the diagnosis of typhoid fever in developing countries can lead to antibiotic abuse. The study aimed to determine sialic acid from the plasma of patients with typhoid fever as alternative for Widal test in diagnosis of typhoid fever. Blood specimens were collected from thirty five (35) adult female and male patients attending the Nigerian Airforce Hospital. The specimens were subjected to typhoid fever diagnosis and sialic acid assay. Incidence of typhoid fever among the patients was determined. Also level of sialic acid was determined among typhoid fever positive and negative patients. Out of the total specimens, 24(68.57%) were found to be infected with *Salmonella typhi* and are set to be the positive group of the study, while 11 (31.43%) patients were not infected with the disease . Incidence of the disease was higher among patients of 17-23 years. Findings of the study shows that there is no significant difference between sialic level of male and female patients but there is a significant difference between the sialic level of *Salmonella typhi* positive and *Salmonella typhi* negative patients. This differences in the level of sialic acid revealed that the infection by *Salmonella typhi* caused increase in the level of total sialic acid (TSA) level in the serum and therefore could be as a diagnostic procedure to determine *Salmonella typhi* infection.

Introduction

Sialic acid, or N-acetyl neuraminic acid, is an acetylated form of the acidic sugar neuraminic acid. It belongs to a family of more than fifty distinct neuraminic acids, often known as sialic acids (Varki 2008). They includes members with different functional groups at the 5-position, for example an *N*-acetyl group, an *N*-glycol group, a hydroxyl group (KDN) or, in the case of neuraminic acid itself, an unsubstituted amine group. Further differences can be found in the position and number of hydroxyl group substituents (for example acetyl, methyl, lactyl, phosphate, sulfate). Diversity in sialic acids may also result due to the opportunity for different glycosidic linkages between the 2-position on sialic acid and other carbohydrate frameworks. Sialic acids are generally found as the terminating unit on glycans, but can also be

found as free molecules, especially in the form of *N*-acetyl neuraminic acid.

Analysis of sialic acid is critical due to its identification as a marker of various disease states. Thus sialic acid, whether naturally found as the monosaccharide or artificially released via chemical or enzymatic means, has previously been identified as a marker for general cardiovascular (CVD) risk and associated diseases such as coronary artery disease (CAD), stroke and diabetes, different cancers and typhoid fever. Sialic acid is overexpressed during the pathogenesis of these diseases and, as such, sialic acid can be indicative of the presence of the disease. Identification and quantification of sialic acid could therefore help in validating sialic acid as a marker for these diseases aiding in diagnosis and more rapid treatment (Cheeseman 2021).

Typhoid fever is a gram- negative bacterial infection caused by the bacterium called *Salmonella typhi (S. typhi),* a rod-like shaped pathogen. *S. typhi* is both food-born and water-born pathogen. Developing nations exhibit a higher incidence of typhoid fever as a result of inadequate sanitation practices and incorrect use of antibiotics, hence fostering the emergence of drug resistance in the microorganisms known as bacteria *S. typhi* (Shanmugan et al., 2025). The diagnosis of this infection is challenging because they can have diverse clinical manifestations with symptoms that overlap with a wide spectrum of other diseases. However, Widal agglutination assay is not sufficiently sensitive, specific, or practical in areas of endemicity (Sulaiman 2006).

Typhoid fever remains a significant public health concern in many regions of the world. Over-dependence on clinical presentation and/or the Widal agglutination test for the diagnosis of typhoid fever in developing countries can lead to antibiotic abuse (Gashaw and Jambo 2022). Sialic acid mediate a wide variety of physiological and pathological processes, serving as ligands for cell adhesion molecules. Due to their negative charge, hydrophobicity and terminal position in sugar chains, they play an important role in cell stability and direct interactions with pathogens and toxins. Research on sialic acid levels in the context of typhoid fever is relatively limited. Sialic acid is over expressed during the pathogenesis of diseases and, as such, sialic acid can be indicative of the presence of typhoid fever. Identification and quantification of sialic acid could therefore help in validating sialic acid as a marker for these diseases aiding in diagnosis and more rapid treatment (Varki 2008; Zhu et al., 2024; Wang et al., 2025). The aim of this research is to determine sialic acid from the plasma of patients with typhoid fever.

Materials and methods

Sample Collection

Blood specimens were aseptically collected from thirty five (35) adult female and male patients between 17-43 years, attending the Nigerian Air force Hospital, located at Airport Road, Kano State, Nigeria. The specimens were subjected to Typhoid fever diagnosis and Sialic acid assay. Incidence of typhoid fever among the patients was determined. Also level of sialic acid was determined among typhoid fever positive and negative (control) patients.

Screening of Typhoid Infection Using Widal test

Two commercially available serodiagnostic Widal kit (ANTEC and Omega Diagnostic Products) febrile kit containing *S. typhi* antigens were used following the manufacturers' instructions. A drop of the suspended antigen was added to an equal amount of serum. Agglutinations were visualized as clumps. Weakly reactive agglutinations were viewed under adequate light source, while strongly reactive agglutinations were easily visible. The results were scored as negative or 0 with no agglutination reaction and the serum that exhibits agglutination at and above ¼ dilutions was considered the end-point titre as described by Olopoenia et al. (2000).

Packed cell volume (PCV) Determination

The percentage of Packed Cell Volume (PCV) in the anticoagulated blood sample was determined according to the micro-hematocrit method (Alexander, 1993) using Hawksley micro-hematocrit centrifuge. The volume occupied by the erythrocytes in the blood is expressed as the percentage of the total volume (packed cell volume, PCV).

The capillary tube was filled to approximately 2/3 with well mixed blood sample. The dry end of the tube was sealed by placing it into the sealant at a 90^0 angle. Capillary tubes were placed in the micro-hematocrit centrifuge with the sealed end toward the periphery. The tubes were at opposite site for balancing in the centrifuge. It was centrifuged for five minutes. Using a micro-hematocrit reading device, the hematocrit was determined.

Sialic Acid Assay

Serum total sialic acid was assessed using Aminoff method by measuring the color intensity in spectrophotometer (Romero et al., 1997). The sample (500µl) was pipetted into a test tube.

Distilled water (500µl) was pipetted into a different test tube as blank. Periodate reagent (250µl) was added to the test tubes. The tubes were incubated at 37^0C for 30 minutes in a water bath. Excess periodate was reduced with 200µl of sodium arsenite reagent. As soon as the yellow color of the liberated iodine disappeared, 2000µl (2ml) of thiobarbituric acid reagent was added and cover the test tubes with aluminium foil. The tubes were incubated in a boiling water bath for 7.5 minutes. The colored solutions was then cooled and shakened with 5000µl (2.5ml) of acid butanol reagent. The tubes were then centrifuged at 3000 x *g* for 5 minutes to facilitate rapid separation of two phases. The colour intensity in the butanol layer was recorded at 549 nm after zeroing the spectrophotometer with the blank.

Results and Discussions

Results

Incidence of Typhoid fever among the patients

Out of the thirty five (35) patients examined, 24 (68.57%) were found to be infected with S.*typhi* and were set as the positive group of the study. Eleven 11 (31.42%) patients were not infected and were as the negative group (negative control) as shown in Table 1 below.

Table 1: Incidence of Typhoid fever among the patients

Number of samples collected	Positive samples (%)	Negative samples (%)
35	24 (68.57)	11 (31.42)

The incidence of Typhoid fever determined by this study is higher than incidences of 36.5% and 47.3% reported by a similar studies in Niger and Ebonyi states in Nigeria (Musa et al., 2025; Ehidiamhen et al., 2025). Another study among typhoid suspected patients in Enugu state reported 5.2% of culture confirmed *S. typhi* which is lower compared to this finding (Ohanu, 2019), Nigeria (14.1%) (Mawazo et al., 2019).

However, the finding from the current study was higher than a report from Nepal (4.1%) (Andrews et al., 2018) (9.2%) (Ohanu, 2019), and India (4.4%) (Bhattacharya et al., 2011) (9.7%) (Mengist and Tilahun, 2017), Egypt (5%) (Srikantiah et al., 2006) of culture confirmed typhoidal *Salmonella* infection. The finding from the current study is higher than studies reported higher proportion of *S. typhi* isolates in South Asia (8.8%) (Zellweger et al., 2017), and Vietnam (14.5%) (Tran et al., 2005). It remains uncertain whether the low incidence and prevalence of *S. typhi* infection in Ethiopia accurately reflects the true burden of the disease or as a result of the inadequate sensitivity of blood cultures. Ethiopia implements mass distribution of azithromycin in regions with significant endemic trachoma; such exposure to

azithromycin may affect sensitivity of blood cultures.

Determination of typhoid fever in relation to age show 5.71% incidence of the disease in patients between 17-23 years with the percentage and 2.86 % incidence in patients between 24-28 years, 2.85 incidence in patients between 29-33 years. 2.85 incidence in patients between 34-38 years, and 2.85 incidence in patients between 39-43 years. This shows that the incidence is higher in patients between 17-23 years. Analysis of the data show that incidence of the disease was significantly related with age of the patients as shown in Table 2. Out of the positives, 48.2% were female and 22.85% were males. Analysis of the data revealed that the incidence was significantly related to gender of the patients (Table 3).

Typhoid fever prevalence was higher among young adults (ages 18–27) and semi-urban residents demonstrating higher infection rates in Niger state, Nigeria (Musa et al., 2025). This is similar to our findings as this study showed higher incidence in patients between 17-23 years. Prevalence of Typhoid fever in Ekiti, South western Nigeria was recorded as 314 males (37.1%) and 532 females (62.9%) giving an M.F ratio 1: 1.9 (Jayeoba et al., 2023). This is similar to the findings of this study. Investigation of a typhoid outbreak in south Tunisia by Hechaichi (2023) reported that over 56% of the infected individuals were male and those under 10 years old were most affected (38.2%% of cases) with a median age of 12 years. According to a study by Xie et al. (2024) in Fujian, China, 23.59% (1 209/5 126) of Typhoid fever cases occurred at the age of 0-4 .The male to female ratio of the cases was 0.97:1.

Table 2: Chi square test result of overall Determination of the Incidence of Typhoid Fever in Individuals of Different age groups in percentage

Age groups	17-23	24-28	29-33	34-38	39-43
Positive (%)	5.71	2.86	2.85	2.85	2.85
Negative (%)	17.14	17.14	22.85	14.28	11.42
Total (%)	22.85	20	25.17	17.14	14.28

X^2 value: 11.09, p value: 0.025

Table 3: Incidence of Typhoid fever in Relation to Patient Gender

Gender	Female	Male
Positives (%)	48.20	22.85
Negatives (%)	20	8.57
Total (%)	68.20	31.42

X^2 value: 0.41, p value: 0.025

Comparison of the PCV mean between the groups show that there is a significant difference between in PCV of patients between 17-23 and 24-28 age groups, there is slight difference between PCV mean of patients between 24-28 and 29-33 age groups, and there is no significant difference between PCV mean of patients between 34-38 and 39-43 age groups (Table 4).

Table 4: Comparison of PCV mean and standard deviation Between Age groups

Age groups (years)	PCV mean ±SD
17-23	35.50 ± 3.92^{a}
24-28	30.85 ± 4.14^{b}
29-33	33.22 ± 4.54^{ab}
34-38	36.33 ± 2.50^{a}
39-43	37.60 ± 3.91^{a}

Key: PCV- Packed cell volume

SD- Standard deviation

Sialic acid Concentration in Typhoid and Non-typhoid patients

The result of sialic acid levels in the serum of typhoid fever patients and non-typhoid patients shows that there is a significant difference between the widal result of typhoid positive and negative patients compared to sialic level. While there is a slight difference in the sialic level compared to gender (Table 5).

A study shows that the levels of total sialic acid TSA (91.58 22.15 mg/dL), LASA (28.88 7.28 mg/dL) and PASA (60.87 15.50 mg/dL) were significantly (P<0.0001) higher in serum typhoid fever patients compared with normal controls (66.0 20.0 mg/dL), (16.0 4.0 mg/dL) and (42.0 7.0) (Hasan et al., 2010).

Table 5: Sialic acid Concentration in Typhoid and Non-typhoid patients

	Categories	Sialic acid concentration (mol/dm^3)
Widal result	Positive	334.96[a]
Gender	Negative	246.30[b]
	Male	314.23[b]
	Female	308.73[ab]

Conclusion

Findings of the study shows that incidence of typhoid fever at the Nigerian Air force Hospital Kano State, Nigeria was higher in females than males among 17-23 years age group significantly related with gender and age. Also, there is a significant difference between the sialic level of *S. typhi* positive and *S. typhi* negative patients. But the level of sialic acid is not related to gender. This differences in the level of sialic acid revealed that the infection by *S. typhi* caused increase in the level of total sialic acid (TSA) level in the serum and therefore could be as a diagnostic procedure to determine *S. typhi* infection.

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Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

Author Contribution

Fatima Hamza Bello - Developing the research idea, conducting experiments and gathering data. Surayya Lawan Idris - Preparing the manuscript, critical reviewing, overseeing the project and mentoring the research idea. Rahama Ismail Ishaq - Revising and reviewing the manuscript. Safiya Inuwa Auwal - Creating tables and performing statistical analysis.

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