Haematological alterations due to typhoid fever in Enugu Urban- Nigeria

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ABSTRACT

Some specific haematological changes that accompany chronic and severe typhoid fever were investigated in an endemic area of Enugu Urban-Nigeria. The results established that typhoid fever infections led to a statistically significant leucopenia ($p < 0.05$). In acute, chronic cases, leucopenia is accompanied with significant oligocythaemia, thrombocytopenia, anaemia and lowered haematocrit ($p < 0.05$). The importance of the results in the diagnosis and treatment of typhoid fever are discussed.

Keywords: typhoid fever, haematological, _Salmonella typhi._

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INTRODUCTION

Typhoid fever is an acute infection of the blood and intestinal systems caused by the bacterium, _Salmonella typhi_ (Turk and Porter, 1982). An individual gets infected with _S. typhi_ which subsequently develops into typhoid fever by swallowing the bacteria (Toohey, 1976). Consequently the bacilli are spread via contaminated food, drink or water. In other words, the disease transmission pathway can be referred to as the "faecal – oral" route. Typhoid fever therefore usually arises due to lack of personal hygiene. It is common in places where there is poor sanitation, but especially where the water supply is liable to be contaminated by human excreta.

Following ingestion, there is an incubation period of about 10 to 14 days (Jawetz _et al._, 1976). At this period the bacteria move from the intestine, through the blood (where they multiply) and spread to the intestinal lymph nodes, gall bladder, liver, spleen etc (Turk and Porter, 1982; Rene and Pines, 2002). However, their main place of settlement is in the intestine where they cause inflammation and ulcers. (Rene and Pines, 2002). Thus there is often a lesion in the intestinal tract with the consequent danger of haemorrhage and intestinal perforation (Turk and Porter, 1982) Perforation of the intestine which is followed by the leakage of the intestinal contents into the abdominal cavity (peritonitis) is a frequent cause of death from typhoid fever. (Rene and Pines, 2002).

Typhoid fever is clinically manifested by a continued fever, headache, constipation (at first) extreme fatigue, joint pain, splenomegaly, inflammation of the intestine with the formation of intestinal ulcers, a characteristic rose-spot eruption on the abdomen, toxaemia etc. (Top, 1968; Jawetz _et al._, 1976, Fuller, 2001) If the typhoid becomes chronic and acute, most of the above symptoms manifest one after the other and may, infact, lead to septicaemia. (Cruickshank _et al._, 1973).

Since the bacilli are found in the blood, faeces and urine of both the ill persons and asymptomatic carriers,

diagnosis may be confirmed by laboratory tests that include isolation of _S. typhi_ from blood, stool and urine specimens and by specific agglutination of _S. typhi_ with the patient's blood serum known as widal reaction (Levine _et al._, 1978; Stephen _et al._, 1984; Pong and Puthucheary, 1989; Schwartz, 2000; Chew _et al._, 1992).

Paratyphoid fever is another human infectious disease similar to typhoid fever caused by some bacteria that closely resemble _S. typhi_ such as _S. enteritidis var paratyphi_ or _var shottmuelleri._

Based on the easy multiplication of _Salmonella_ spp in the blood stream, this paper aims to determine precisely some specific haematological changes that accompany acute and chronic typhoid fever in Enugu Urban where there is evidence of the disease prevalence. (Umeh, 2004) The information obtained may serve as useful guidelines in arriving at a better diagnosis and treatment of the disease in endemic areas such as Enugu and elsewhere and whilst carrying out further related studies.

MATERIALS AND METHODS

From January 6, 2006 to January 16, 2007 172 blood samples were collected from different adult individuals who came to St. Rita's laboratory at Emene – Enugu to be screened of both typhoid fever and Malaria parasites respectively.

By the use of 5 mL disposable syringes about 3 mL of blood were drawn from the veins of the arms of each person and transferred into 5ml test tube which already contained 0.05 g of potassium oxalate as anti-coagulant.

About 1 mL of this blood was used to screen for typhoid fever and malaria parasites using the widal test and light microscope respectively (Levine _et al._, 1978; Stephen _et al._, 1984; Pong and Puthucheary, 1989; Chew _et al._, 1992) Only the blood samples of 19 patients who had typhoid fever without malaria were used for further tests. Typhoid positive serum was taken as that

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with visible agglutination at $\geq$ 1:160 dilution. (Levine *et al.*, 1978; Nwaugo *et al.*, 2005).

Blood samples were also got from 119 student volunteers of Enugu State University of Science and Technology (ESUT) Enugu who did not complain of symptoms of malaria or typhoid fever. However 1ml each of their blood samples were also screened for typhoid fever and malaria parasites respectively and only 68 of those samples that had neither typhoid nor malaria were used as control for typhoid.

From about 2 mL of blood remaining in each blood sample (typhoid and non-typhoid) the RBC, WBC and platelet counts as well as haemoglobin contents and haematocrit values were determined, following the methods of Baker and Silverton (1982).

Thus, a very small quantity of each blood sample was diluted with one of the RBC diluting fluids, Formol citrate (sodium citrate 3.0 g; Formaldehyde 1.0 mL; and distilled water 100.0 mL) in the ratio of 1:250. Thus by the use of a haemoglobin pipette, 0.02 mL of blood were drawn from the test tube and washed into 5.0 mL of formol citrate contained in a bijou bottle.

The mixture was loaded into a special glass counting chamber, the improved Neubauer haemocytometer. The erythrocytes were allowed to settle for 5 min before their number was counted with the aid of light microscope. Duplicate counts were made for each blood sample and the mean taken. The number of erythrocytes was calculated and recorded (Baker and Silverton, 1982).

The second part each blood sample was diluted with Turk's solution (1% glacial acetic acid) in the ratio of 1:20. Thus 0.05 mL of blood was drawn with a haemoglobin pipette from a test tube into 1.0 mL of Turk's solution contained in a bijou bottle. This mixture was loaded into the improved Neubauer haemocytometer, already used for erythrocytes. The number of leucocytes present in ruled areas meant for counting only the leucocytes was recorded. The leucocyte number was calculated and recorded (Baker and Silverton, 1982).

The third part of each blood sample was diluted with 1% ammonium oxalate in the ratio of 1:20. Again, by the use of a haemoglobin pipette, 0.02 mL of blood were drawn from the test tube and washed into 0.4 mL of 1% ammonium oxalate contained in a bijou bottle. The mixture was loaded into the Improved Neubauer haemocytometer used for erythrocytes and leucocytes. The platelets were allowed to settle for 5 min before their number was counted with the aid of phase contrast microscope. Duplicate counts were made for each blood sample and the average taken. The number of platelets was calculated and recorded (Baker and Silverton, 1982).

About 1.0 mL of the remaining blood sample was drawn into a wintrobe microhaematocrit tube which was graduated from 0 to 100mm and filled up to the 100 mm mark. The tube was centrifuged at 3000 revolutions per minute in a Gallenkamp centrifuge for 30 min. The height of the resulting column of packed blood cells was read from the millimeter reading graduations and expressed as the percentage of blood cells within the whole blood or packed cell volume (PCV) (Wintrobe, 1961).

A small volume of the remaining sample of blood was diluted with modified Drabkin's fluid (Potassium cyanide 0.2 g; Potassium ferricyanide 0.2 g; Sodium bicarbonate 1.0 g and the whole mixture was made up to 1 L of solution using distilled water) in the ratio of 1:250. In other words 0.02 mL of the blood sample was mixed with 5.0 mL of Drabkins fluid of pH 7.0. Then about 1.0 mL of this mixture was poured into a cuvette and its optical Density (O.D) read in a photoelectric colorimeter at absorbance of 540 nm, zeroing with blank. The haemoglobin content of each blood sample was calculated following the methods of Hester and Girling (1991) and Chinyere and Nwachukwu (2001).

$$Hb4 = \frac{T \times C \times D}{A \times 1000} g/100mL$$

Where

T	=	test absorbance at 540nm
A	=	standard absorbance at 540nm
C	=	concentration of cyanmethaemoglobin standard (mg/100mL).
D	=	Dilution factor = 250
1000	=	converts mg/100mL to g/100mL.

RESULTS

The mean differences between haematological values obtained for typhoid patients were compared with those of non-typhoid individuals. It was found that typhoid fever led to a statistically significant leucopenia ($p < 0.01$), which is in the reduction of White Blood Cells (WBC) Red Blood Cells (RBC) Blood Platelet counts as well as haemoglobin contents and PCV ($p < 0.05$) (Tables 1 and 2)s In more severe and chronic cases, leucopenia is accompanied with thrombocytopenia ($p<0.05$) oligocythaemia ($p<0.05$) hypohaemoglobinaemia ($p <0.05$) and reduced haematocrit ($p<0.05$).

DISCUSSION

The significant leucopenia commonly observed in typhoid fever is attributed to invasion of haemopoietic organs such as lymph nodes, spleen, tonsils, bone marrow etc by *S. typhi* which radically slowed down the rate of leucopoiesis. The invasion of the above organs by *S. typhi* which can also depress the rate of haematopoiesis may also explain the observed oligocythaemia and thrombocytopenia seen in typhoid patients. Thrombocytopenia results in generating spontaneous bruises and prolonged bleeding lesions in the intestinal tract with the consequent danger of haemorrhage and intestinal perforations.

The observed oligocythaemia and hypohaemoglobinemia tend to explain why most acute and chronic typhoid fever cases usually lead to iron deficiency anaemia. (Lucas and Gilles, 1989).

Table 1: Haematological data observed amongst 68 non-typhoid persons in Enugu Urban, Nigeria.

Sp	RBCC	WBCC	B.PC	Hb$_4$C	Hct	Sex
A$_1$	5.6	8.0	250,	16.5	51	M
B$_1$	5.2	9,5	300,	14.6	45	M
C$_1$	5.5	7.0	180,	17.4	53	M
D$_1$	5.4	9.0	280,	15.3	46	M
E$_1$	4.5	10.5	380,	13.8	42	F
F$_1$	5.7	9.5	320,	15.4	47	M
G$_1$	5.5	9.0	300,	14.8	45	M
H$_1$	4.9	5.0	250,	13.6	41	F
I$_1$	4.8	10.5	350,	13.9	42	F
J$_1$	5.1	8.0	150,	14.0	43	F
K$_1$	4.9	10.0	350,	13.5	41	F
L$_1$	5.0	10.5	280,	16.5	50	M
M$_1$	4.8	8.5	330,	13.8	43	F
N$_1$	5.5	9.0	500,	14.2	44	F
O$_1$	5.1	11.0	450,	16.8	52	M
P$_1$	6.0	8.0	340,	14.6	44	M
Q$_1$	5.8	9.2	200,	15.7	48	M
R$_1$	5.4	9.5	350,	14.5	44	F
S$_1$	4.7	9.0	300,	14.0	41	F
T$_1$	5.3	9.0	250,	14.8	45	F
U$_1$	4.8	9.0	400,	13.9	43	F
V$_1$	4.7	8.5	380,	13.8	42	F
W$_1$	4.5	8.5	350,	13.6	41	F
X$_1$	4.8	10.0	400,	14.1	40	F
Y$_1$	5.5	10.5	420,	15.5	49	M
Z$_1$	5.9	11.0	400,	15.9	50	M
A$_3$	5.8	10.5	350,	15.8	51	M
B$_3$	5.1	10.0	360,	15.1	51	M
C$_3$	5.4	9.5	350,	15.4	51	M
D$_3$	4.4	8.5	280,	13.6	46	F
E$_3$	4.6	8.0	250,	13.7	47	F
F$_3$	5.3	9.0	350,	15.3	51	M
G$_3$	5.1	9.0	330,	15.1	50	M
H$_3$	4.9	8.5	310,	15.0	49	M
I$_3$	5.8	10.0	350,	15.8	51	M
J$_3$	4.7	8.0	280,	14.9	52	M
K$_3$	5.7	9.5	400,	15.7	54	M
L$_3$	5.6	9.0	280,	15.6	53	M
M$_3$	5.9	9.5	320,	16.2	53	M
N$_3$	5.8	9.0	240,	16.1	52	M
O$_3$	6.0	10.0	500,	16.4	54	M
P$_3$	5.0	8.0	300,	14.6	45	M
Q$_3$	5.1	8.5	280,	14.8	45	M
R$_3$	5.4	8.5	350,	15.4	46	F
S$_3$	4.7	7.0	250,	14.0	44	F
T$_3$	5.5	11.0	300,	15.5	55	M
U$_3$	4.9	8.0	240,	14.5	42	F
V$_3$	5.5	8.5	330,	14.8	48	M
W$_3$	5.3	8.0	320,	15.5	53	F
X$_3$	5.7	9.5	330,	15.7	57	M
Y$_3$	5.6	9.0	310,	15.6	56	M
Z$_3$	5.5	9.5	300,	15.5	53	M
A$_5$	4.7	9.0	200,	14.3	41	F
B$_5$	4.9	8.5	350,	14.4	43	F
C$_5$	4.6	8.0	300,	14.1	42	F
D$_5$	5.1	10.5	320,	14.6	44	F
E$_5$	5.5	10.0	350,	15.5	43	M
F$_5$	5.6	9.5	360,	15.6	41	M
G$_5$	5.8	9.0	38,	15.8	46	M
H$_5$	5.0	9.0	260,	14.4	45	F
I$_5$	4.9	8.5	270,	14.5	44	F
J$_5$	4.8	10.0	250,	14.1	43	F
K$_5$	4.7	8.5	300,	13.9	45	F
L$_5$	4.9	9.5	300,	14.2	46	F
M$_5$	5.3	10.5	350,	13.8	47	F
N$_5$	5.2	10.8	340,	13.9	44	F
O$_5$	4.7	9.2	280,	13.6	40	F
P$_5$	5.5	11.0	500,	15.4	45	M

Sp	=	Specimen
RBCC	=	Red Blood Cell Count in millions per mm^3
WBCC	=	White Blood Cell Count in thousands per mm^3
BPC	=	Blood Platelet Count in thousands per mm^3
Hb$_4$C	=	Haemoglobin contents in gm/dL
Hct	=	Haematocrit (.%)

Table 2: Haematological data observed amongst 51 typhoid patients in Enugu Urban, Nigeria.

Sp	RBCC	WBCC	B.PC	Hb4C	Hct	Sex
A$_2$	4.3	3.6	130,	10.2	31	M
B$_2$	3.5	2.9	120,	9.6	30	F
C$_2$	3.4	3.7	180,	8.8	29	F
D$_2$	4.1	3.4	100,	10.6	32	M
E$_2$	3.9	3.8	95,	10.4	32	M
F$_2$	3.8	3.6	90,	10.6	34	M
G$_2$	3.8	3.5	110,	8.5	29	F
H$_2$	3.6	3.0	140,	9.7	30	F
I$_2$	4.4	2.6	135,	11.0	35	M
J$_2$	4.0	3.0	95,	9.9	30	M
K$_2$	3.6	2.2	120,	9.0	29	F
L$_2$	4.1	2.8	115,	10.1	32	M
M$_2$	3.6	3.0	80,	9.4	29	F
N$_2$	3.9	2.5	120,	10.3	33	M
O$_2$	3.7	2.9	125,	8.5	28	F
P$_2$	3.5	2.6	110,	8.8	29	F
Q$_2$	4.2	3.4	105,	10.4	35	M
R$_2$	3.9	3.2	100,	9.8	32	M
S$_2$	3.2	2.5	100,	8.3	28	F
T$_2$	3.3	2.8	105,	8.9	29	F
U$_2$	3.1	3.0	85,	9.1	27	F
V$_2$	3.5	1.8	80,	8.8	31	F
W$_2$	4.1	2.4	120,	10.1	32	M
X$_2$	3.9	2.2	125,	10.3	35	M
Y$_2$	3.8	2.6	135,	10.5	34	M
Z$_2$	3.8	3.4	130,	10.3	35	M
A$_4$	4.2	2.2	115,	10.2	33	M
B$_4$	3.6	2.6	85,	9.2	29	F
C$_4$	3.1	2.0	100,	9.8	28	F
D$_4$	3.4	2.5	90,	9.1	30	F
E$_4$	3.3	2.0	95,	8.9	33	F

Sp	$RBCC$	$WBCC$	BPC	Hb_4C	Hct	Sex
F_4	3.5	2.4	90,	9.0	31	F
G_4	3.4	3.0	110,	11.2	26	F
H_4	4.0	2.6	130,	12.8	37	M
I_4	4.1	1.8	125,	10.5	36	M
J_4	4.2	2.1	130,	10.1	35	M
K_4	3.6	2.4	105,	9.3	34	F
L_4	3.9	2.6	120,	10.8	36	M
M_4	3.8	2.8	80,	9.5	30	F
N_4	3.2	2.0	95,	8.7	34	F
O_4	4.0	2.5	115,	12.8	40	M
9_4	4.1	2.4	125,	11.5	36	M
Q_4	3.5	2.3	90,	9.0	30	F
R_4	3.2	2.4	105,	9.2	30	F
S_4	2.9	2.7	95,	9.6	29	F
T_4	3.3	3.0	115,	9.4	29	F
U_4	3.0	2.6	105,	8.9	32	F
V_4	3.1	3.3	100,	9.1	30	F
W_4	2.8	2.9	108,	8.4	31	F
X_4	4.4	3.1	125,	10.6	37	M
Y_4	4.0	3.2	130,	10.2	35	M

Sp	=	Specimen
RBCC	=	Red Blood Cell Count in millions per mm^3
WBCC	=	White Blood Cell Count in thousands per mm^3
BPC	=	Blood Platelet Count in thousands per mm^3
Hb_4C	=	Haemoglobin content in mg/dL
Hct	=	Haematocrit (.%)

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