

Asian Journal of Medical Sciences 7(3): 26-29, 2015

ISSN: 2040-8765; e-ISSN: 2040-8773

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Submitted: January 19, 2015

Accepted: February 14, 2015

Published: July 25, 2015

## Assessment of Lipid Profile of Enteric Fever Patients in Enugu Metropolis, South-East Nigeria: Useful or Useless?

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**Abstract:** Enteric fever has been implicated in complications such as severe sepsis and in alteration of some biochemical and hematological parameters. Enteric fever affects the intestine, which is also the site for lipid absorption, but its possible effect on lipid metabolism is unclear. The present study was aimed at estimating the lipid profile of enteric fever patients in Enugu metropolis. Lipid profile of 200 enteric fever patients and 100 apparently healthy subjects in Enugu metropolis were determined using standard techniques. Enteric fever was investigated using rapid slide titration method and the confirmatory test done with Enterocheck-WB<sup>®</sup> kit. Serum Total Cholesterol (TC), Triacylglycerol (TG), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) were assayed using standard operative procedures. Statistical analysis was done with graph pad prism computer soft ware using student's t-test to test for statistical significance. *P*-values of <0.05 were considered to be statistically significant. The lipid profile of all the patients showed non-significant difference ( $p > 0.05$ ) when compared with the control. However, when the male and female subjects were separately analyzed, TC and LDL-C significantly increased ( $p < 0.05$ ) in the male subjects compared to the male controls, whereas the female subjects showed significant decrease ( $p < 0.05$ ) compared to the female control. The study suggests that lipid profile is not significantly altered in enteric fever infection and thus may be useless in enteric fever management.

**Keywords:** Apoproteins, hyperlipidemia, total cholesterol, typhoid infection

### INTRODUCTION

The bacterial genus *Salmonella*, was firstly isolated from animal intestine (Crum, 2003). *Salmonella* comprises of two species, *Salmonella bogori* (*S. bogori*) and *S. enterica*. Of all the subspecies of *Salmonella enterica*, only subspecies *enterica* is associated with disease in worm blooded animals such as man (Kimbrough and Miller, 2000). *Salmonella* is divided into distinct serologic groups (A through E) on the bases of their somatic O-antigens (Amal and Eman, 2010; Pang *et al.*, 1998).

The agents that cause enteric fever are therefore *Salmonella enterica* subspecies *enterica* serovar typhi

and serovar paratyphi (A, B and C) (Amal and Eman, 2010). Although Paratyphi-A and typhi cause a similar illness, with relapsing fever, paratyphi-A generally causes a milder disease (Parry *et al.*, 2006; McClelland *et al.*, 2004).

Enteric fever is a major public health problem in developing countries of the world with an estimated annual incidence of 540 per 100,000 individuals (Katung, 2000). Enteric fever is endemic in the economically disadvantaged countries in Africa and has also been shown to be more severe in infants and the elderly (Preston and Borezyk, 1994; Gatsing *et al.*, 2006). Symptoms of enteric fever include high fever, constipation, extreme fatigue, head ache, joint pain and

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rash across the abdomen as rose spots (Lesser and Miller, 2001).

Lipids are known to play important roles in virtually all aspects of biological life, providing energy storage and metabolic fuels serving as hormone precursors acting as functional and structural components of bio-membranes, aiding digestion and forming insulation to allow nerve conduction or prevent heat loss (Brites *et al.*, 1998; Burtis *et al.*, 2001). Hyperlipidemia, therefore, refers to the increase in the plasma concentrations of triacylglycerols (TGs) or cholesterol, both exposing the body to the dangers of formation of arteriosclerotic plaques with hypertriglyceridemia being more common (Murray *et al.*, 2003).

Complications of enteric fever include liver and spleen enlargement, anaemia, intestinal bleeding, joint heart and brain infection and these lead to severe sepsis which is known to alter the lipid profile (Khosla *et al.*, 1991). The effect of enteric fever in the stomach and intestines may also affect the lipid absorption and lead to alteration in the plasma lipid concentrations of such patients, hence the need for the present study.

The general objective of the present study is to determine the effect of enteric fever on lipid profile of enteric fever patients in Enugu metropolis. The result of this study will help in the diagnosis and disease-treatment monitoring of enteric fever patients in our locality.

## MATERIALS AND METHODS

**Study setting:** The study was conducted at the Department of Chemical Pathology, University of Nigeria Teaching Hospital, (UNTH) Ituku-Ozalla, Enugu State.

**Study population:** The subjects comprised of a total of 300 adults (170 females and 130 males) with the age groups of 20-40 years. Two hundred patients (120 females and 80 males), recruited from the University of Nigeria Teaching Hospital UNTH Ituku-Ozalla, ESUT Teaching Hospital G.R.A, Enugu, Ntasiobi-ndi-non'afufu Hospital Enugu and Annunciation Hospital, Emene Enugu, made up the test subjects. The severity of the disease was evaluated by the severity of abdominal pain, high fever, extra-intestinal manifestations of the disease and general well being. The control subjects were 100 apparently-healthy subjects (50 females and 50 males), recruited from the same locality of the hospitals. Informed consent was obtained by all the subjects and ethical clearance obtained from the Hospitals before the commencement of the study.

**Inclusion criteria:** Subjects were included in the study if they were diagnosed with enteric fever, with symptoms such as abdominal pain, high fever and extra-intestinal manifestations of the disease.

**Exclusion criteria:** All the subjects having malaria infection, diabetes mellitus, hypertension, obesity and renal problems were excluded from the study.

**Specimen collection and processing:** Five mls of fasting blood samples were collected from the subjects via a clean vene-puncture from the median cubital vein into a clean, labeled plain tube. The fresh blood samples were immediately tested for typhoid fever while the rest of the samples were allowed to clot and retract and then centrifuged at 3000 rpm for 5 min using desk-top centrifuge (Bran Scientific Co England ®). The sera were separated from the clot and deep-frozen at -20°C and used within 5 days of collection.

## ANALYTICAL METHODS

**Screening test for enteric fever:** Rapid slide titration method was used to carryout the widal test on all the blood samples, (Kimbrough and Miller, 2000) using commercial antigen suspension. Samples with positive widal test results (high antibody titre from O 1/160 and H 1/160 and above) were further tested using Enterocheck-WB ® kit for confirmation and subjected to lipid profile test.

**Enteric fever confirmatory test:** Enterocheck-WB Kit form Zephyr Biomedicals India which was sensitive to the study environment, was used for confirmatory test. Enterocheck-WB utilizes the principle of immunochromatography. The kit components were brought to room temperature and placed on flat horizontal surface. Exactly 5 µL of serum sample was dispensed into the specimen port "A" whereas 5 drops of running buffer was added into the reagent port "B" and the result read at the end of 15 mins.

**Lipid profile estimation:** Enzymatic estimation TC was done by the method of Allain *et al.* (1974) whereas the method of Fossati and Principe (1982), was used for enzymatic estimation of TG. Precipitation/enzymatic method of Allain *et al.* (1974), Burstein *et al.* (1970) and Groove (1979) was used for HDL-Cholesterol estimation. All the kit reagents were produced by Biosystem S.A ® Barcelona (Spain). The LDL and VLDL were both estimated in the serum using the Friedewald formula (Friedewald *et al.*, 1972).

**Statistical analysis:** Data entry and statistical analysis utilized the graph pad prism computer soft ware. Data was analyzed using student's t-test to test for statistical significance. *P*-values of <0.05 were considered to be statistically significant.

## RESULTS

Table 1 represents the lipid profile (mmol/L) of enteric fever patients and control. The table showed a

Table 1: Lipid Profile (MMOL/L) among enteric fever patients and control

Lipid profile parameters	Patients (n = 200)	Controls (n = 100)	p-value
Triacylglycerides (TG)	1.4±0.47	1.32±0.31	0.0904
Total Cholesterol (TC)	4.54±1.15	4.49±0.95	0.6694
HDL-C	1.19±0.39	1.24±0.31	0.2168
LDL-C	2.73±1.03	2.65±0.82	0.5365
VLDL-C	0.64±0.21	0.60±0.40	0.056

Table 2: Lipid profile of male patients and age-matched controls

Lipid profile parameters	Patients (n = 80)	Controls (n = 50)	p-value
Triacylglycerides (TG)	1.37±0.42	1.27±0.31	0.1598
Total Cholesterol (TC)	4.74±1.56*	4.22±0.92	0.0343
HDL-C	1.25±0.42	1.22±0.34	0.7878
LDL-C	2.87±1.34*	2.42±0.71	0.029
VLDL-C	0.62±0.49	0.57±0.14	0.159

\* = statistically significant compared to the control

Table 3: Lipid profile of female patients and age-matched controls

Lipid profile parameters	Patients (n = 120)	Controls (n = 50)	p-value
Triacylglycerides (TG)	1.42±0.49	1.38±0.31	0.5655
Total Cholesterol (TC)	4.41±0.75*	4.77±0.89	0.009
HDL-C	1.16±0.36	1.26±0.26	0.008
LDL-C	2.62±0.74*	2.90±0.86	0.0381
VLDL-C	0.65±0.22	0.62±0.14	0.4221

\* = statistically significant compared to the control

non-significant ( $p < 0.05$ ) decrease in HDL-C and increase in the rest of the parameters in the test subjects when compared with the controls. The total cholesterol and LDL-C however, showed a significant increase ( $p < 0.05$ ) in the male test subjects when compared to the male controls (Table 2). A comparison between the female patients and control revealed a significant decrease ( $p < 0.05$ ) in LDL-C and total cholesterol in the test subjects (Table 3).

## DISCUSSION

Lipid profile has been known to be altered in patients with severe sepsis and few studies regarding the status of lipid levels in enteric fever show that the analysis of the lipid profile may be essential in making a diagnosis (Levy *et al.*, 2000). In the present study we tested the hypothesis that plasma lipid profile may be altered in patients with enteric fever. The results showed no statistically significant difference ( $p < 0.05$ ) in the studied lipid profile parameters of the overall patients (males and females), when compared to the control (Table 1).

The observed result is not in agreement with the study by Khosla *et al.* (1991) which reported elevated levels of LDL-C and triacylglycerides in acute enteric fever patients in India. The observed difference may be attributed to difference in the patient population from different geographical locations, environmental factors and nutritional lifestyle (NCEP, 1993). It may also be as a result of methodologic differences or difference in severity of the disease, as the patients in the study by

Khosla *et al.* (1991) were patients with complications of enteric fever such as severe sepsis which they reported, lead to alteration in lipid profile.

Furthermore, there was a significant increase in the total cholesterol and LDL-C of the male patients compared to control, whereas in female patients, a significant decrease ( $p < 0.05$ ) was recorded in total cholesterol and LDL-C of the patients compared to the control. This sex-related increase in total cholesterol and LDL-C in males may be as a result of decrease in concentration of LDL-receptors in the liver, (Burtis *et al.*, 2001) since LDL-receptors help in the removal of LDL-C from the circulation. It could also be as a result of reduced LDL binding due to defective LDL receptors.

Typhoid fever characterized by increased chronic inflammatory cells infiltrates in the mucosal lesions. The excessive local production of soluble mediators from activated monocytes and polymorphonuclear leukocytes has been implicated in mediating the tissue injury (Weiss, 1989). Important among these mediators are oxygen free radicals.

Disturbances in lipoproteins resulting from peroxidative attack may affect their normal metabolism and the subsequent distribution of both lipid and vitamin moieties to peripheral organs (Amal and Eman, 2010). The increased total cholesterol and LDL-C of male patients when compared with female is in agreement with the report of NCEP (1993) that before menopause, women tend to have lower total cholesterol compared to men of the same age-range. Menopause, however, is associated with increased LDL-C in post-menopausal women (Ikekepeazu *et al.*, 2009).

Although the present study showed that the increased LDL-C and total cholesterol levels may be sex-influenced/sex-dependent, the concurrent increase in total cholesterol and LDL-C in the present study may be attributed to the fact that cholesterol is a major constituent of LDL-C, in addition to apoproteins (Burtis and Ashwood, 1999).

## CONCLUSION

The present study showed that there was no significant alteration in the concentration of serum lipids of enteric fever patients. Lipid profile estimation in such population may not be used in the diagnosis or treatment monitoring of enteric fever.

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