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LDL-CHOLESTEROL SERUM VARIATIONS RELATE TO SERUM HOMOCYSTEINE IN FEMALE PATIENTS WITH ISCHEMIC STROKE

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ABSTRACT

Ischemic stroke occurs due to lack or interruption of blood supply to brain mainly due to blockade of a blood vessel. The present report provides further information about the concentration and interrelationship of LDL cholesterol (LDL-C) and homocysteine (Hcy) in normal healthy women and women patients with ischemic stroke. Clinical diagnosis and management of the patients was carried out and complete history was recorded. The LDL-C, Hcy and other physiological/ biochemical measurements were carried out by kit methods mainly ELISA kits. The LDL-C serum levels (mg/ dl) in female patients with ischemic stroke and normal healthy female controls were 110.45± 2.09 (n:24) and 89.05±2.88 (n:21) respectively. Statistical comparison showed highly significant variations ($p< 0.0001$). Serum Hcy levels (µmol/L) in female patients with ischemic stroke and normal healthy female controls were 9.33±0.54 (n:24) and 7.74±0.35 (n:21) respectively. Statistical comparison showed highly significant variations ($p = 0.0161$). The Hcy plotted against LDL-C did not show any significant linear relationship in normal healthy women but a highly significant linear correlation (slope: 3.3191; $R^2 =0.7457$; p-value= 4.63E-7) was found in women patients with ischemic stroke. The present study provides interesting investigations and will hence be helpful for carrying out further studies especially to understand the beneficial role of factors for reducing LDL-C and Hcy for better management of patients with ischemic stroke and related disorders.

Key-words: Serum LDL-cholesterol, homocysteine, ischemic stroke

INTRODUCTION

Stroke is a neurovascular or cerebrovascular disorder in which poor blood flow to brain leads to cellular injury and death. Ischemic stroke occurs owing to lack or interruption of blood supply to brain mainly due to blockade of a blood vessel.

Increased tHcy (total Hcy) serum levels were found associated with increased risk of strokes in middle men (Virtanen *et al.*, 2005). The literature reveals that serum levels of LDL cholesterol (LDL-C) might elevate in response to cerebral ischemia (He *et al*., 2017). Furthermore, Women with ischemic stroke were found to have elevated LDL-C levels compared to men (Zhang *et al*., 2014).

There are several factors and conditions that may reduce the levels of LDL-C and/ or Hcy in patients with ischemic stroke (Mierzecki *et al*., 2013; Lee *et al*., 2018).

Increased levels of Hcy may cause increase in LDL-C (Mierzecki *et al*., 2013). Men and women patients with family history of PIS (premature ischemic stroke) showed significantly higher LDL-C, and Hcy in men associated with higher LDL-C (Mierzecki *et al*., 2013). Hyperhomocystenemia was found in 60.6% and elevated LDL-C in 10.8 % stroke patients with median age of 56 years and having risk factors of diabetes, hyperlipidemia, smoking, obesity and family histrory (Kalita *et al*., 2009).

Genetic studies reveal that although PON (paraoxonase) gene polymorphisms and ischemic stroke did not show any association, PON1L55 allele was found associated with plasma Hcy and PON2S311S and PON2G148 G alleles with LDL-C plasma concentrations (Shin *et al.*, 2008).

It was tried pharmacologically to decrease LDL-C and Hcy which influenced the ischemic condition in male and female patients with family history of PIS (premature ischemic stroke), low level of physical activity, hypertension, obesity and smoking (Mierzecki *et al*., 2013). Apolipoprotein E (*APOE*) gene study in stroke patients under exercise training suggested decrease in LDL-C and Hcy (Lee *et al*., 2018). Furthermore, women whose parents had ischemic stroke showed higher levels of LDL-C (Mierzecki *et al*., 2005).

Our previous work is quite helpful for studying ischemic stroke (Khan and Hussain, 2009; Khan *et al*., 2009; Naz *et al*., 2009;) and other related studies (Hussain, 1991; Fatima *et al*., 2007; Hussain *et al*., 2007; Yasmeen *et al*., 2008; Javaid *et al*., 2012; Sohail and Hussain, 2013; Javaid *et al*., 2019).

The present study does not provide gender based evidence for change in LDL-C and / or Hcy in ischemic stroke, post-ischemic stroke, and in ischemic stroke under the influence of conditions/ factors that may decrease LDL-C and / or Hcy. However, it provides information about the change in LDL-C and Hcy and their interrelations occurring in women patients with ischemic stroke. It is important to mention that interrelationships and correlation between LDL-C and Hcy in patients with ischemic stroke have rarely been studied using well controlled data. Hence, this study is a significant step in clarifying the role of interrelationships and correlation between LDL-C and Hcy in patients with ischemic stroke.

MATERIALS AND METHODS

Total number of normal control women subjects (n:24) and women with ischemic stroke (n:21) were included in the present study. Age range of the subjects was 55-65 years. Some of the preliminary clinical assessments and biochemical/physiological tests were performed in subjects and patients to decide which specific parameters be investigated thoroughly.

The patients with ischemic stroke were first diagnosed properly with the help of their clinical manifestations as well as biochemical/physiological assessments, and differential diagnosis was performed with wider clinical perspectives. Proper diagnosis needed family and medical history of the patients and thorough examination including physical check up and performing diagnostic/biochemical tests. In that regard, expert clinicians helped in diagnosing the patients with ischemic stroke.

A variety of tests including clinical, neurological, biochemical, physiological, radiographic, ultrasound and nuclear imaging diagnostic tests were found helpful for diagnosing patient with ischemic stroke. Another very important aspect was related to involvement of risk factor/factors (e.g. hyperlipidemia, smoking, hypertension, diabetes, obesity etc). The Patients with ischemic stroke having single or mixed risk factors were consulted.

The clinical, physiological and biochemical study of these subjects/ patients was done thoroughly. About 10 ml of blood was drawn. The blood sample was centrifuged quickly to avoid false increase of Hcy levels due to its release from RBCs. Samples were divided into required number of aliquots and refrigerated at specific temperatures for various tests until used.

The CED984Ge96 tests kit (ELISA kit for Hcy) was also used wherein inter-assay variations (<13%) and intra-assay variations (<11%) were noticed. The assay (competitive inhibition (between biotin-labelled Hcy and un-labelled Hcy) enzyme-immunoassay method incorporating monoclonal-antibody for Hcy precoated upon microplate) was carried out by preparing samples, standards and reagents, by taking 50 µl sample or standard in each well with the immediate addition of 50 µl of detection reagent (shaking, mixing and one hour incubation at the temperature of 37°C, aspirating and washing three times, addition of 100 µl of detection reagent with incubating half time than the previous and at same temperature, aspirating five times), then adding 90 µl substrate solution with incubation of about 20 minutes at 37°C and adding 50 µl stop solution and then immediately reading at 450 nm.

Colorimetric (enzymatic) procedure involved cholesteryl-ester hydrolase, cholesterol-oxidase and peroxidase respectively for hydrolyzing cholesteryl esters, producing hydrogen peroxide and a dye for the formation of coloured substance. For LDL-C, the cholesterol other than LDL-C was measured and subtracted from total cholesterol that gave the concentration LDL-C. The automated procedure for LDL-C was also used in some of the initial pilot experiments for comparing the results by manual and fully automated methods. In the fully automated procedure, LDL-C in serum was determined by combining sugar-compound with the detergent, both measured photometrically (absorbance: 585nm). For the estimation of LDL-C, we used other procedures in the pilot studies. Abcam's kit (ab65390) was used for separating HDL from LDL cholesterol. The principle was that cholesterol oxidase recognizes specifically the contents of cholesterol and forms the chemicals that by reacting with probe give colour at 570 nm and fluorescence as Ex/Em which is 538/587. Resultantly, the free cholesterol and ester of cholesterol are separately detected.

The MS Excel was used for the data entry, and descriptive statistics was employed for comprehensive analysis. The p value of < 0.05 was specified as significant statistically. The one way ANOVA was incorporated for

comparing various groups. Values for F crit, p and F were determined and the r^2 was found for linear regression lines. Spreadsheets (written for Excel; workable with Calc program) were found helpful for analyzing the data. Using spreadsheets was easy compared to statistical analysis in SAS or SPSS.

The Y intercept, regression coefficient, the r^2 value, degree of freedom, the P value and *Y* estimator and an *X* estimator were obtained with the help of spreadsheets.

Serum level of LDL cholesterol (LDL-C ; mg/ dl) and homocysteine (Hcy; µmol/L) were determined in normal healthy women. These controls served for comparing the results of the patients with ischemic stroke. The results were compared statistically applying general principles (Zahir *et al*., 2014).

RESULTS

The LDL cholesterol (LDL-C) serum levels (mg/ dl) in female patients with ischemic stroke and normal healthy female controls in Mean ± SEM were 110.45± 2.09 (n:24) and 89.05±2.88 (n:21) respectively. Statistical comparison (unpaired t-test) showed highly significant variations (t = 5.8704, p value < 0.0001).

Serum homocysteine (Hcy) levels (µmol/L) in female patients with ischemic stroke and normal healthy female controls in Mean ± SEM were 9.33±0.54 (n:24) and 7.74±0.35 (n:21) respectively. Statistical comparison (unpaired t-test) showed highly significant variations (t = 2.5043, p value = 0.0161).

To find correlation between serum levels of Hcy and LDL-C in normal healthy subjects, Hcy levels were plotted against LDL-C levels (Fig.1). It showed a highly non-significant linear relationship (Slope: 1.4867; R^2 =0.0332; p-value= 0.394).

Correlation between Hcy and LDL-C levels in patients with ischemic stroke was found by plotting Hcy levels against LDL-C levels (Fig.2). Results showed a highly significant linear correlation (Slope: 3.3191; R^2 =0.7457; p-value= 4.63E-7).

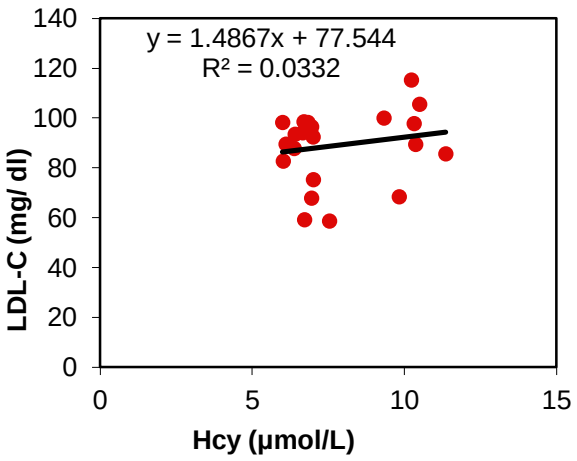

Fig.1. Correlation between serum levels of homocysteine and LDL cholesterol in normal healthy subjects.

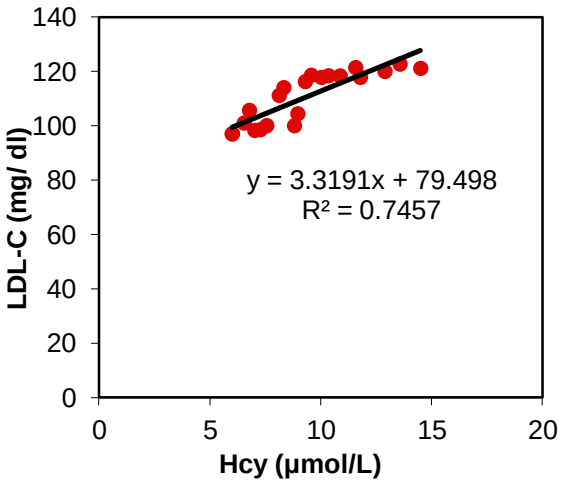

Fig.2. Correlation between serum levels of homocysteine and LDL cholesterol in patients with ischemic stroke.

DISCUSSION

The present report is interesting information about the concentration and interrelationship of LDL-C and Hcy in normal healthy women and women patients with ischemic stroke. Several studies were carried out previously for collecting and interpreting similar data (Kalita *et al*., 2009; Virtanen *et al*., 2005; Mierzecki *et al*., 2013; Zhang *et al*., 2014; He *et al*., 2017; Lee *et al*., 2018). However, the precise interrelationship of the correlation between LDL-C and Hcy in normal healthy women as well as women patients with ischemic stroke was not evident.

We investigated significant elevation in LDL-C and Hcy serum levels in women patients with ischemic stroke in our present study. This part of our report is quite similar to previous reports (Kalita *et al*., 2009; Virtanen *et al*., 2005; He *et al*., 2017). However, information obtained from plot between Hcy and LDL-C serum levels and investigation of their correlation for interrelationship in normal healthy women as well as women patients having ischemic stroke is not available employing well controlled data.

The present work will hence, be helpful for carrying out further studies especially to understand the beneficial role of factors for reducing LDL-C and Hcy for better management of patients with ischemic stroke and related disorders.

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