Available online at

ScienceDirect
www.sciencedirect.com

Elsevier Masson France

EM|consulte
www.em-consulte.com

Original Article

Assessment of liver function in pregnant anemic women upon oral iron and folic acid supplementation

A.K.M. Tiwari [a,b], A.A. Mahdi [a,*], S. Mishra [b]

[a] Department of Biochemistry, King George's Medical University, Shahamina Road, Lucknow 226003, India
[b] Department of Biotechnology, School of Engineering & Technology, IFTM University, Moradabad, India

ABSTRACT

Oral iron therapy is the most widely prescribed treatment for iron deficiency anemia. However, oral iron supplementation may also lead to various health problems. The recognition of these physiological variations is essential for the diagnosis of liver diseases during the course of pregnancy. Therefore, the objective of this study was to assess the variations in levels of routine liver function tests (LFTs) in pregnant women before and after iron and folic acid treatment. Iron and folic acid was supplemented to 500 normal pregnant anemic women (mild = 200, moderate = 200 and severe = 100) and 100 age matched normal pregnant non-anemic as controls daily for 100 days. Blood index values and liver function parameters were precisely monitored. Hemoglobin (Hb), total protein (TP), iron (Fe), albumin and alkaline phosphatase (ALP) levels were found increased ($P < 0.001$; $P < 0.01$; $P < 0.05$) after treatment in mild, moderate, severe and control, respectively. Lipid peroxidation (LPx), aspartate transaminase (AST) and alanine transaminase (ALT) were increased in pretreated mild, moderate and severe groups and further increased after all treated subjects. Moreover, gamma-glutamyl transpeptidase (GGT) was found to decrease in pre and posttreated subjects. Treatment with iron and folic acid although has remarkable efficacy for Hb and body iron stores although for the cost of increasing the associated compartment of total bilirubin, AST and ALT concomitant with decreased GGT levels. Data obtained from the present study provide new insights into the mandatory application of liver function tests likely to be monitored at regular and specific intervals during the course of pregnancy.

© 2017 Published by Elsevier Masson SAS.

Introduction

Iron deficiency anemia is one of the most commonly encountered medical disorders during pregnancy, affecting more than two billion people worldwide, with pregnant women at particular risk [1]. In developing countries, it is a cause of serious concern as, besides many other adverse effects on the mother and the fetus, it contributes significantly high maternal mortality [2]. Iron deficiency can lead not only to anemia but may also impair work performance, lead to an abnormal neurotransmitter function and ultimately result in altered immunological and inflammatory defences [3]. Moreover, women tend to have substantially low iron stores, enabling them more vulnerable to iron deficiency as and when iron intake is lowered or necessitates increases. Oral iron therapy is the most widely prescribed treatment for iron deficiency anemia (IDA); however, there are many issues, probably revealing

a controversy referring to oral iron supplementation from successfully managing IDA. For instance, many patients do not respond adequately to oral iron therapy due to difficulties associated with ingestion of the tablets and their side effects, probably playing a significant role in rates of compliance [4,5]. However, oral iron supplementation may also lead to various health problems due to its increased bioavailability in the body. The deleterious effect of excess iron is related to its ability to generate free radicals [6]. It has been earlier suggested that oral iron supplementation may increase its availability in the intestine and thus may participate in fenton reaction leading to production of an excess of reactive oxygen species resulting in local oxidative stress [5,7].

There are certain reports stating a subclinical physiological cholestasis during the phase of pregnancy [8]. The augment in plasma volume that occurs during pregnancy leads to hemodilution [9,10] and ultimately reduces the serum protein concentrations. Serum alkaline phosphatase levels have been noticed to increase in late pregnancy [11,12]. It does, thus, not astonish that change in liver function tests (LFTs) occurs during pregnancy.

Nevertheless, except for increased alkaline phosphatase levels, have been clearly established, the variations in the other LFT values have not been clearly monitored. Besides, an overview in this concern has clearly reflected certain discordances in the literature [13]. The recognition of these physiological variations is essential for the diagnosis of liver diseases during the course of pregnancy.

Therefore, the present study was undertaken to assess the variations in plasma levels of routine LFTs before and after iron and folic acid treatment, i.e., total protein (TP), albumin, total bilirubin (TB), alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and gamma-glutamyltranspeptidase (GGT) in anemic pregnant women compared with a control group of non-anemic pregnant women.

Materials and methods

Subjects

The study was approved by the Institutional Ethical Committee of King George's Medical University, Lucknow, India. Before recruitment, written informed consent was obtained from each subject. In total, four groups of subjects were selected for the study. Out of 600, 100 were pregnant non-anemic women as control and 500 were pregnant anemic (mild = 200, moderate = 200 and severe = 100) women as cases. Selected subjects were all consumers of normal mixed Indian diet, not taking any drugs for preceding one month. The inclusion criteria of anemic subjects were according to WHO, which defines mild anemia as Hb 10.0–10.9 g/dL, moderate as Hb 7.0–9.9 g/dL and severe as Hb < 7.0 g/dL [14]. Women who have been using mineral and/or vitamin supplements were also excluded. Patients, which did not show Hb rise by 1% after 3 week of supplementation, were also excluded from the study. Both groups were non-smokers, non-alcoholic and without any other symptoms known to influence the antioxidant status and minerals. Information on occupation and medical history, job description, socio-economic status and lifestyle of both groups were obtained through questionnaires. Subjects having a previous history of metabolic diseases such as hypertension, diabetes mellitus, malignancy, heart disease, infections such as tuberculosis, HIV and arthritis and endocrinal disorders were also excluded from the study.

Treatment

At recruitment, all women (non anemic and anemic) were first dewormed by giving them a single dose of albendazole following metronidazole (400 mg) three times daily for five days. Two days later, i.e. after one week of recruitment, women were given iron supplements (100 mg as ferrous sulphate and 500 μg folic acid) orally once a day, daily for 100 days.

Before deworming, venous blood of all women (non-anemic and anemic) was taken for the estimation of biomarkers of blood indices and trace minerals (pretreatment). All tests were repeated after 100 days of treatment (posttreatment). After three weeks of iron and folic acid supplementation, Hb was assessed. Subjects, who's Hb was not improved, were excluded from the study and were referred for other investigations. All blood collections were done in the Department of Obstetrics & Gynaecology, Queen Mary's Hospital, King George's Medical University, Lucknow. The subjects were instructed not to change their dietary or daily activities during the study.

Sample collection

Blood samples were collected in the Queen Mary's Hospital, King George's Medical University, Lucknow. At enrollment and after hundred days of iron and folic acid supplementation, 6 mL venous blood was taken from each of the subjects and divided into three aliquots. One milliliters of blood was transferred to an evacuated tube containing sodium citrate solution used to determine hemoglobin (Hb). Two milliliters of whole blood was also transferred into sodium citrate containing tube and then centrifuged; plasma separated and used for the estimation of Fe, lipid peroxide level (LPx) and total protein (TP). Remaining 3 mL of venous blood was also centrifuged at 3000 rpm for 15 min, serum separated and used for the estimation of albumin, total bilirubin (TB), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and gama-glutamyltranspeptidase (GGT).

Analytical estimation

Blood hemoglobin was determined by using the cyanome-themoglobin method [15]. The concentration of iron in plasma was measured with flame atomic absorption spectrophotometer (Perkin Elmar AAS-700 Ueberlinger, Germany) [16]. The total protein (TP) levels were measured by the method of Lowry et al. [17]. The total protein of the sample were estimated spectrophotometrically at 660 nm and expressed as mg/dL. The lipid peroxidation (LPO) levels were measured by the method of Okhawa et al. [18]. The thiobarbituric acid reacting substances (TBARS) of the sample were estimated spectrophotometrically at 532 nm and expressed as nmole of MDA/mg protein. Serum ALP activity was measured using the method of Bessey et al. [19]. Serum ALT activity was measured using the method of Henry et al. [20]. Serum GGT activity was measured by Szasz's colorimetric method [21]. Serum levels of total and conjugated bilirubin were measured by Jendrassik and Grof's method [22]. Serum albumin level was measured by Rodkey's method [23]. All of the methods described were adapted for automated analysis (SMAC Technicon). Serum AST activity was measured by a technique derived from the technique of the Société française de biologie clinique [24].

Statistical analysis

Healthy pregnant women (control) and pregnant anemic women (mild, moderate and severe) before and after treatment were compared together using one-way ANOVA analysis of variance by Neuman–Keules post hoc test between groups. A probability P-value of < 0.05 ($P < 0.05$) was considered statistically significant. The statistical analysis was performed on commercial software INSTAT 3.0, a demo version (Graph Pad Software, San Diego, CA).

Results

Blood parameters

General characteristics of pregnant women highlighted in Table 1 were considered mandatory prior to studying blood parameters during the present investigation.

The pre and posttreatment blood Hb, Fe and lipid peroxidation parameters of all pregnant healthy and anemic women are summarized in Table 2. The posttreatment mean values of blood parameters in control were found to be increased significantly ($P < 0.001$). However, the pretreatment mean values of all blood parameters in all anemic women were found to be lower as compared to the respective control except lipid peroxidation value. Treatment of these anemic women with elemental iron and folic acid (100 mg and 500 μg/day for hundred days) showed a significant reversal of above parameters. Treatment significantly increased the blood Hb in mild (10.49 ± 0.23 mg/dL vs 12.18 ± 0.59 mg/dL), moderate (8.03 ± 0.54 mg/dL vs 10.70 ± 1.20 mg/dL)