Available online at http://www.ijirr.com

International Journal of Information Research and Review
Vol. 2, Issue, 09, pp.1137-1141, September, 2015

IRR INTERNATIONAL JOURNAL

OPEN ACCESS JOURNAL

ISSN: 2349-9141

Full Length Research Paper

ESTIMATION OF SERUM VITAMIN B12 LEVELS IN METABOLIC SYNDROME PATIENTS: A TERTIARY HOSPITAL BASED STUDY IN EASTERN PART OF INDIA

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Received 27th August 2015; Published 30th September 2015

Abstract

Introduction: The Metabolic Syndrome, a collection of five problems namely abdominal obesity, hypertension, hyperglycemia, increased triglyceride and decreased level of HDL cholesterol, greatly increase a person's risk for stroke, heart attack and diabetes. On other side, vitamin B12 has a great protective role on haematological, neurological and cardiovascular system.
Aims: To estimate serum vitamin B12 levels among the patients attending hospital with metabolic syndrome and find out the relation of serum vitamin B12 with the various biochemical parameters of metabolic syndrome patients.
Materials and Methods: This cross sectional, observational, case-control, and hospital based study included metabolic syndrome patients as cases and age-sex matched healthy subjects as controls. Weight, height, and hip & waist circumferences and blood pressure were measured in cases and control group. Biochemical parameters such as lipid profiles (total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and triglyceride), diabetic profiles (fasting & postprandial glucose and HbA1c) and vitamin B12 levels were assayed in both groups.
Results: Enrolled in the study were 100 metabolic syndrome patients and 100 age-sex matched healthy control. In socio-demographic data; weight, BMI, waist circumference and waist-hip ratio were strongly related between two groups. In clinical data analysis, blood pressures (systolic and diastolic) were statistically related between cases and controls. Biochemical parameters i.e. lipid profile (total cholesterol, HDL, LDL, VLDL, triglyceride) and diabetic profile (FBS, PPBS, HbA1c) were recorded and found strong statistical relation between metabolic syndrome patients and controls. The results of vitamin B12 levels of both two groups gave strong statistical significance (in cases, 161.0 ± 97.3 pg/ml; in controls, 312.88 ± 119.7 pg/ml).
Conclusion: The present study revealed that there is significant decrease of vitamin B12 level among metabolic syndrome patients when it is compared with normal healthy population.

Keywords: Vitamin B12, Metabolic Syndrome.

***To cite this paper**:* Saumyajit Maiti and Kamal Lochan Das, 2015. Estimation of serum Vitamin B12 levels in Metabolic Syndrome patients: a tertiary hospital based study in eastern part of India*, International Journal of Information Research and Review.* Vol. 2, Issue, 09, pp.1137-1141, September, 2015.

INTRODUCTION

The metabolic syndrome (MetS) is, a clinical condition, clustering components of metabolic derangements such as abdominal obesity, insulin resistance, hyperinsulinemia, impaired glucose tolerance, dyslipidemia, hypertension, and a proinflammatory and prothrombotic state. (Reaven, 1988) Presence of MetS indicates over nutrition, sedentary life style, and resultant excess adiposity. (Marc-Andre Cornier *et al*., 2008) It is a common cause of development of atherosclerotic vascular disease and type 2 diabetes. (Isomaa *et al*., 2001) It is estimated that around 20-25 per cent of the world's adult population have the metabolic syndrome and they are twice as likely to die from and three times as likely to have a heart attack or stroke compared with people without this syndrome. Moreover, people with metabolic syndrome have fivefold greater risk of developing type 2 diabetes. (Sterne *et al*., 2004)

Though this MetS was more prevalent in the industrial and developed countries, but in the progress of time, it is observed the prevalence of metabolic syndrome is increasing day by day very rapidly in the developing countries like India. Vitamin B_{12} or cobalamin, a water soluble vitamin and one of the most essential micronutrients, has a great role in DNA synthesis. Vitamin B_{12} has also an important participation in haemopoesis, neurocognitive and cardiovascular functions (Oh *et al.*, 2003). In the normal adults, the total amount of stored vitamin B_{12} in the body is about 2-5 mg, out of which approximately the half of the previous amount is stored in liver. (Oda, 2009; Quadri *et al.,* 2004) Biochemical and clinical vitamin B12 deficiency presents with diverse clinical manifestations ranging from impaired memory, dementia, delirium, peripheral neuropathy, sub-acute combined degeneration of the spinal cord, megaloblastic anemia and pancytopenia. Vitamin B_{12} deficiency is found in pernicious anaemia or inadequate intake of Vitamin B_{12} through diet,

especially in those who are vegetarian. The research works have already been done over a decade to find out the relation between several parameters of metabolic syndrome and serum Vitamin B_{12} level in the human body, in the whole over the globe. But it has been observed that the results obtained by some researchers differ from those of others on this topic. So, in our study, we aimed to evaluate the serum Vitamin B_{12} levels together in patients with MetS and to find whether these values are related with the several anthropometric, clinical & biochemical components of MetS patients in the eastern part of India.

Aims and Objectives

In this study, we aimed to evaluate the plasma Vitamin B_{12} levels together in patients with MetS and to find out whether these values are related with the several components of metabolic syndrome.

MATERIALS AND METHODS

Study Design

This was a hospital-based, observational, cross-sectional, case-control study. The study was conducted at Hi-Tech Medical College and hospital, Bhubaneswar, India by the Department of Biochemistry in collaboration with the Department of Medicine over a period of one year (from January 2013 to December 2013). One hundred cases were recruited from the patients of metabolic syndrome, attending outpatient department (OPD) of Department of Medicine of Hi-Tech Medical College & Hospital for treatment. Age-sex matched another one hundred healthy controls were recruited from relatives and peers of the patients, persons attending OPD for routine health check-ups, and from staffs & faculties of Hi-Tech Medical College & Hospital. The study design included a prospective component as biochemical evaluations were carried out once, in a single hospital visit.

After obtaining informed consent, patients of Department of Medicine were screened for inclusion & exclusion criteria. 100 cases among them were selected for the study. Each of them was subjected to physical examination followed by a brief questionnaire. Then 10ml of blood was drawn under proper aseptic precaution of each of the patient. 100 controls, age & sex matched, were selected from the OPD & among staffs & faculties of the college. Each of them was also subjected to physical examination followed by the same questionnaire and 10ml of blood sample was drawn.

Definition of the Metabolic Syndrome

The definition of the metabolic syndrome was recommended by the International Diabetes Federation (IDF). According to new IDF definition, for a person to be defined as having the metabolic syndrome they must have

- Central obesity (defined as waist circumference with ethnicity specific values) [If BMI is >30kg/m², central obesity can be assumed and waist circumference does not need to be measured.]
- Plus any two of the following four factors:
- Raised triglycerides: ≥ 150 mg/dl (1.7 mmol/l) or specific treatment for this lipid abnormality
- Reduced HDL cholesterol (HDL-C): < 40 mg/dl (1.03 mmol/l) in males, < 50 mg/dl (1.29 mmol/l) in females or specific treatment for this lipid abnormality
- Raised blood pressure: systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension
- Raised fasting plasma glucose (FPG): (FPG) ≥ 100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes. If above 5.6 mmol/L or 100 mg/dL, Oral glucose tolerance test (OGTT) is strongly recommended but is not necessary to define presence of the syndrome.

Inclusion criteria for cases

- Known metabolic syndrome patients on regular check-up.
- Known diabetic patients with central obesity and hypertension.

Exclusion criteria for cases

- Suffering from deficiency of Vitamin B_{12} or taking any sorts of medicines to improve serum Vitamin B_{12} levels.
- Patients with coronary heart disease, significant valvular disease, life-threatening systemic disease, chronic obstructive pulmonary disease.
- Suffering from any chronic debilitating disease like malignancy, acute infection, trauma.
- Suffering from any chronic liver ailments or renal disorders.
- Pregnant women.

Criteria for Control

Apparently healthy subjects or persons attending OPD for routine health check-up with age group of 40-60 years.

Parameters study

Demographic Parameters – Age, Sex
Anthropometric Parameters - Waist circumference, Hip circumference, Height, Weight
Blood Pressure - Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP)

Biochemical Parameters

Diabetic Profile (serum Fasting Blood Sugar [FBS], serum Post-prandial Blood Sugar [PPBS], glycosylated Haemoglobin [HbA1c]), Lipid Profile (serum Total Cholesterol [TC], serum Triglyceride [TG], serum High Density Lipoprotein Cholesterol [HDL-C], serum Low Density Lipoprotein Cholesterol [LDL-C], serum Very Low Density Lipoprotein Cholesterol [VLDL-C]), and serum Vitamin B_{12} levels.

Biochemical Analysis

All of the blood and urinary samples were collected in the morning, after 8-12 h of overnight fasting. After blood was drawn, the tubes were gently shaken and then separated by centrifugation at 3200 rpm for 10-15 min. A blood sample for postprandial glucose level was drawn 2 h after eating. FBS, PPBS, and lipid profile were estimated by ERBA 200 automatic analyser, only low density lipoprotein-cholesterol

(LDL-C) was calculated using the Friedewald formulation. HbA1c were determined by Bio-Rad D-10 Dual Program Automatic Analyser and serum Vitamin B_{12} levels were estimated by solid-phase chemiluminescent immunometric assay.

Methods for Collection of Other Data

A pre-designed, pre-tested, semi-structured questionnaire was used to collect various socio-demographic data like name, age, sex, address along with data about physical examination and clinical history. Heights were measured in centimetre scale using a stadiometer; fraction values were approximated to the nearest centimetre. Weights were taken in kilograms using a calibrated weighing machine and fractions were approximated to its nearest kilogram. Body Mass Index (BMI) or the "Quetelet Index" was calculated as per the formula of Adolphe Quetelet. Blood pressure (BP) was measured (in mm of Hg) using a mercury sphygmomanometer and a standard cuff in the arm. The average of three measurements was recorded as final recording.According to the World Health Organization's data gathering protocol, (STEPS, 2015) the waist circumferences were measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a stretch-resistant tape that provides a constant 100 g tension. Hip circumferences were measured around the widest portion of the buttocks. In these measurements, the tape was parallel to the floor. For both measurements, the individual was stood with feet close together, arms at the side and body weight evenly distributed, and little clothing to be put on. The subjects were relaxed, and the measurements were taken at the end of a normal expiration.

Each measurement was repeated twice; if the measurements were within 1 cm of one another, the average was calculated. If the difference between the two measurements exceeds 1 cm, the two measurements were repeated (Waist Circumference and Waist-Hip Ratio, 2015). Waist-Hip Ratio (WHR) is used as a measurement of obesity, which in turn is a possible indicator of other more serious health conditions. WHO STEPS states that abdominal obesity is defined as a waist–hip ratio above 0.90 for males and above 0.85 for females, or a body mass index (BMI) above 30.0 (http://whqlibdoc.who.int/publications/2011/9789241501491_eng.pdf, 2015).

Statistical Methods

The data collected was checked for error, cleaned and double entered into MS-Excel spread sheets and checked for any entry error. Then the whole data was imported into IBM SPSS Statistics (version 20.0) and further analysis was done. Data was first summarized and then analyzed for test of significance e.g. chi-square test, independent sample student t-test wherever applicable using the software package. P value less than 0.05 was taken as significant. The whole procedures involved were transcription, preliminary data inspection, content analysis and interpretation.

Ethical Issues

There was no associated risk or chance of harm to study subject other than the minimal risk associated with phlebotomy. However phlebotomy was done using disposable syringe & needle with complete aseptic measures and under direct supervision to minimize risks. Every subject was observed for an hour for any complication after phlebotomy.

The protocol of the study was approved by the Institutional Ethics Committee (IEC) for Human Research, Hi-Tech Medical College & Hospital, Bhubaneswar. Voluntary informed consent was taken from all participants in a consent form that was reviewed & approved by the IEC. Consents were taken by principal researcher himself. All participants were clearly explained that they reserve the right to withdraw from the study at any time they choose. A copy of the consent form was given to each participant. The study was continuously being monitored by the IEC during the study period. No internal/ external funding/ grant were received for the study. It was solely funded by principal investigator himself. No conflict of interest was there to be declared.

RESULTS

A total 100 middle aged metabolic syndrome patients and 100 age-sex matched healthy controls were included in this study. Some socio-demographic features, anthropometric and clinical parameters of MetS patients and control group and their comparison are shown in Table 1. Table 1 is showing the distribution of age and sex between case and control groups. Among the MetS cases, 66 of them were females and remaining 34 were males. Female to male ratio was 1.94:1. The mean (±SD) age of cases was 45.9 (±5.1) yrs. Among the controls, 68 were females and 32 were males; female to male ratio was 2.1:1. The mean (±SD) age of control group was 46.2 (±5.3) yrs. Chi-square test shows the difference in male and female distribution between both groups were statistically insignificant (P= 0.687); similarly the difference in age

Table 1. Comparison of socio-demographic, anthropometric and clinical parameters of metabolic syndrome patients and control group

Parameters	Metabolic Syndrome (n=100)	Control Group (n=100)	p Value
Age (mean years ± SD)	45.9 ± 5.1	46.2 ± 5.3	Ns
Male	34	32	Ns
Female	66	68	Ns
Female : Male	1.94 : 1	2.1 : 1	Ns
Height (mean cm ±SD)	164.2 ± 8.7	166.5 ± 9.3	Ns
Weight (mean kg ± SD)	80.7 ± 9.3	66.4 ± 11.5	<0.001
BMI [mean (kg/m^2) ± SD]	29.6 ± 1.5	22.8 ± 1.3	<0.001
Waist circumference (mean cm ± SD)	91.4 ± 7.3	79.9 ± 4.2	<0.001
Hip circumference (mean cm ± SD)	101.1 ± 7.1	98.4 ± 5.3	Ns
Waist-Hip Ratio (mean ± SD)	0.9 ± 0.03	0.8 ± 0.04	<0.001
SBP (mean mm of Hg ± SD)	136.7 ± 7.1	126.6 ± 6.1	<0.001
DBP (mean mm of Hg ± SD)	85.6 ± 5.0	82.0 ± 5.4	=0.003

distribution between groups were also insignificant (P= 0.832). Height, weight, waist circumference (WC), and hip circumference (HC) were recorded for each subject. Body mass index (BMI) and waist-hip ratio (WHR) were calculated from collected data. BMI was calculated by [weight in kilogram / (height in meter)^2]. Then the data was summarized by calculating the mean and SD for each parameter in both the group differently.

Table 1 is also showing the summarized data on anthropometric parameters and blood pressure for both groups. The means of the both case and control groups were compared and tested by independent sample t test for statistical significance. The mean height of the controls were slightly higher than cases, but it is not significant (P= 0.949). Slightly larger hip circumference was seen among cases, but this was also not significant (P= 0.192). However, significantly higher weight (P< 0.001), BMI (P< 0.001), waist circumference (P< 0.001), and waist-hip ratio (P< 0.001) were observed among cases in comparison to the control group. SBP and DBP were recorded for each subjects of both case and control groups.

Above table is showing the summarized data on blood pressure for both groups. Among the cases, 82 were on anti-hypertensive therapy, but 18 and total control group did not take any antihypertensive drugs.

The means of SBP and DBP of the both case and control groups were compared and tested by independent sample t test for statistical significance. Significantly higher SBP (P<0.001) and DBP (P= 0.003) were observed among cases in comparison to the control group. Various cutoff values for vitamin B_{12} have been propounded. Currently, there is no certain agreement on the clinical use of cutoff levels for vitamin deficiency. A recent widely used clinical cutoff level for vitamin B_{12} deficiency was 200 pg/ml (148 pmol/l) (Allen, 2009). Various biochemical parameters of metabolic syndrome and serum vitamin B_{12} were assessed and the result is given below in Table 2. The serum lipid profile was estimated for both cases and controls.

Among the cases, 68 were on the medication to improve the lipid profile, and rest 32 of cases and the total control group did not take any such medication. Table 2 is showing the summarized data of lipid profile of case and control groups and their comparison. The differences in various parameters of lipid profile were checked for statistical significance by independent sample t test.

The mean of serum total cholesterol, HDL-C, LDL-C, VLDL-C, and TG – all the parameters of lipid profile are more in cases than controls and are statistically strongly significant (all p value <0.001) between cases and controls. The diabetic profile was estimated in both case and control groups. Here the diabetic profile is consists of FBS, PPBS, and HbA1c. In serum FBS, the mean (±SD) were 125.7 (±10.9) mg/dl in cases and 115.7 (±6.7) mg/dl in controls. In case of serum PPBS, the mean (±SD) were 154.3 (±30.4) mg/dl and 135.8 (±6.9) mg/dl in the cases and control group respectively. The summarized data of diabetic profile of case and control groups is shown in Table 2.

The anti-diabetic drugs were taken by 78 of the cases, whereas the remaining 22 and total control group did not take any sort of anti-diabetic drugs. Again the differences in parameters of diabetic profile were checked for statistical significance by independent sample t test. Here, the mean of serum FBS & PPBS and HbA1c – all the parameters of diabetic profile are more in the cases than controls and are statistically strongly significant (all p value <0.001) between cases and controls.

The serum vitamin B_{12} levels were assessed in both groups; in MetS patients and control group. In metabolic syndrome patients, the mean (±SD) of serum vitamin B_{12} level were 161.0 (±97.3) pg/ml, whereas in control group, the mean (±SD) of serum vitamin B_{12} level were 312.88 (±119.7) pg/ml. The means of two groups are compared by independent sample t test for statistical significance and that shows a strong statistical relation (P <0.001).

Table 2. Comparison of biochemical parameters of metabolic syndrome patients and control group

Parameters	Metabolic Syndrome (n=100)	Control Group (n=100)	p Value
Lipid Profile:			
Total Cholesterol (mg/dl)	210.6 ± 41.9	173.9 ± 4.8	<0.001
HDL-C(mg/dl)	42.3 ± 6.5	53.3 ± 3.9	<0.001
LDL-C (mg/dl)	124.2 ± 27.3	91.6 ± 3.2	<0.001
VLDL-C (mg/dl)	48.2 ± 25.7	23.7 ± 2.9	<0.001
Triglyceride (mg/dl)	227.0 ± 121.7	125.9 ± 10.5	<0.001
Diabetic Profile			
FBS (mg/dl)	125.7 ± 10.9	115.7 ± 6.7	<0.001
PPBS (mg/dl)	154.3 ± 30.4	135.8 ± 6.9	<0.001
HbA1c	7.2 ± 0.42	6.2 ± 0.13	<0.001
Vitamin B_{12} (200pg/ml)	161.0 ± 97.3	312.88 ± 119.7	<0.001

The mean (±SD) of serum total cholesterol was 210.6 (±41.9) mg/dl among cases in contrast to 173.9 (±4.8) mg/dl among controls. The mean (±SD) of serum HDL-C was 42.3 (±6.5) mg/dl in the case group whereas 53.3 (±3.9) mg/dl in the control group. The mean (±SD) of serum triglyceride among the cases and controls were 227.0 (±121.7) mg/dl and 125.9 (±10.5) mg/dl respectively. The mean (±SD) of serum LDL-C among the cases and controls were 124.2 (±27.3) mg/dl and 91.6 (±3.2) mg/dl respectively. And the mean (±SD) of serum VLDL-C was 48.2 (±25.7) mg/dl among the cases in contrast to 23.7 (±2.9) mg/dl in the control group.

DISCUSSION

In the present study, vitamin B_{12} levels were reduced in middle-aged MetS patients when it is compared with the control group. There were some limitations in this study. Firstly, users of Proton Pump Inhibitors (PPIs) from both MetS and control groups were not excluded in our study, as use of PPIs might be effective at reduction of vitamin B_{12} levels in human beings. Secondly, homocysteine (tHcy) levels of metabolic patients and control group were not estimated here. In several researches, it was observed that there was a relation

between homocysteine and vitamin B_{12} concentrations. Third, we did not exclude the state of H. pylori infection in case and control groups. Previous studies suggested that there was a relation between vitamin B_{12} deficiency and H. pylori manifestations.

Summary and Conclusion

In the literature, some studies evaluated vitamin B_{12} levels in obese patients, with MetS or without, and tried to draw a relationship between vitamin B_{12} deficiency and obesity. They concluded different results in their studies. Vitamin B_{12} levels were normal in some studies, whereas levels were low in others. Uehara and Rosa observed that cobalamin levels were normal in MetS patients in their study (Uehara *et al.,* 2008). Guven *et al*. also got the same kind of result (Guven *et al.,* 2005). Jermendy *et al*. Noticed normal tHcy and vitamin B_{12} levels in their study (Jermendy *et al.,* 2001). Again Tungtrongchitr *et al.,* 2013 assessed serum vitamin B_{12}, homocysteine, and folic acid levels in overweight and obese subjects and they concluded no statistically significant difference in the vitamin B_{12} levels compared with normal weight subjects (Tungtrongchitr *et al.,* 2005). Reitman *et al*. conducted a study on plasma vitamin B_{12}, antioxidants, and homocysteine levels in patients with obesity. They concluded no significant difference in vitamin B_{12} levels between the obese and lean subjects in their results (Reitman *et al.,* 2002).

However, Karatela *et al*. conducted a study on vitamin B_{12} and homocysteine levels in hypertensive overweight and obese compared to hypertensive normal-weight patients. It was reported a high level tHcy and reduced vitamin B_{12} level in their study (Karatela, 2009). Moreover, Pinhas-Hamiel *et al*. conducted a study on obesity in children and adolescents and found that obesity was associated with vitamin B_{12} deficiency (Pinhas-Hamiel *et al.,* 2006). A study by Davut Baltaci *et al*. got reduced levels of vitamin B_{12} in metabolic syndrome patients of Turkey (Davut Baltaci *et al.,* 2012). The present study revealed that there is significant reduction of vitamin B_{12} level among MetS patients. However, larger study with better sample size is required for further exploration. Moreover, we should estimate the level of vitamin B_{12} in metabolic syndrome patients with prime importance and start the necessary treatment as early as possible.

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