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Effect of dietary deficiency of vitamin B_6, vitamin B_{12} and folic acid on the development of coronary artery disease in adult Indian male patients

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Abstract: *The present study was conducted to find out the correlation of dietary deficiency of vitamin B_6, vitamin B_{12} and folic acid on development of coronary artery disease (CAD). This study was carried out on 172 male patients of age group between 45-65 years having both genetic and non-genetic background for CAD from Kolkata, India. Present study showed that BMI, SBP, DBP, LDL-c, triglyceride, total cholesterol, fasting and post prandial blood glucose level, homocysteine, CPK and CPK-MB enzyme levels were higher while HDL-c, LVEF and hemoglobin levels were lower than recommended normal values in both subject groups. Daily consumption of carbohydrate, vitamin B_{12}, vitamin B_6 and folic acid levels of both subject groups were found to be lower while consumption of fat was higher than recommended values. Present study showed that there was a significant correlation between intake of vitamin B_6, vitamin B_{12} and folic acid and risk factors for CAD in both groups. All these findings indicate that folic acid, vitamin B_6 and vitamin B_{12} deficiency may have influence in developing risk for CAD.*

Key words: *Coronary artery disease, folic acid, risk factors, vitamin B_6, vitamin B_{12}*

I. Introduction

The Coronary artery disease (CAD) has become a major public health problem in many developing countries. Epidemiological data reveal that CAD caused 7 million deaths in 2010 that increased from 5.2 million in 1990 globally [1]. It is expected that prevalence of CAD in India may increase upto 111% by 2020 as compared to 77% for China, 106% for other Asian countries and 15% for developed countries [2]. Presently plasma homocysteine has been identified as an important risk factor for CAD. Several reports showed that transformation of homocysteine from methionine is blocked by vitamin B_6, vitamin B_{12} and folic acid. Accumulation of homocysteine in blood resulting from the deficiency of these vitamins reduces oxidation of LDL-cholesterol (CAD risk factor) and also causes endothelial injury making it susceptible to the accumulation of lipids and deposition of them, thus confers the independent risk of CAD [3]. Besides, elevated level of serum cholesterol, triglycerides, diabetes, high blood pressure, family history, age, sex, diet, sedentary life style, stress, obesity, physical practices such as smoking, alcohol consumption are the traditional risk factors for CAD. CAD also involves both genetic and environmental factors as well as interaction between them [4, 5]. Moreover, it is suggested that elevated level of creatine phosphokinase (CPK) and cardiac-specific CPK (MB isoenzyme) indicate the myocardial infarction [6].

On the other hand, it is reported that individuals may be affected from CAD at any age but CAD has become dramatically more common at progressively older ages. In addition to this men are reported to be more susceptible to CAD than the women [7, 5]. Moreover, it is also suggested that persons having family history of cardiac disease and obesity have a tendency to develop CAD [7]. In this context we had an interest to study the correlation of dietary deficiency of vitamin B_6, vitamin B_{12} and folic acid on development of CAD of hospitalized male patients of age 45-65 years from Kolkata, India having both genetic and non-genetic background for CAD.

II. Materials And Methods

2.1 Data collection

The present study was conducted on the patients admitted to Birla Heart Research Hospital and Research Centre and Royd nursing home, Kolkata with the diagnosis of coronary artery disease (CAD). To carry out the present study, total 172 male patients of age group between 45-65 years having only CAD were selected from 250 randomly selected patients through the self reported questionnaire method to get information regarding age, family history, life style including physical activity levels and food habits for at least five years prior to diagnosis, life practices such as smoking, alcohol consumption etc. Patients whose first line relatives (i.e., mother, father, sister and brother) suffered from or died of CAD were considered as a subject group with

genetic background of CAD (Group I). On the other hand, patients whose first line relatives did not suffer or die of CAD were considered as a subject group with non-genetic background of CAD (Group II). On the other hand, CAD patients suffering from cancer, respiratory diseases, kidney diseases or any other complications and also patients suffering from other cardiac diseases were excluded from the present study. Besides, before getting admitted to the hospital the CAD patients who were under medications such as folic acid, vitamin B_6 and vitamin B_{12} those interfere with the metabolism of plasma homocysteine were also excluded from this study. Thus, following the above mentioned inclusion criteria eighty five patients were selected in Group I and eighty seven patients were selected in Group II. This study was conducted following the human ethical guidelines and all data were collected with the informed consent from the patients and hospital authority.

Subjects having sedentary life style were selected according to their daily energy expenditure [8]. Height, weight, systolic blood pressure (SBP) and diastolic blood pressure (DBP) and left ventricular ejection fraction (LVEF) values of all subjects were collected from their hospital records just after admission but before onset of medication and treatment of CAD. Body mass index (BMI) of each patient was further calculated using respective height and weight data by standard method [9].

2.2 Laboratory Data

All blood estimations of the subjects were conducted by collecting fasting blood from the subjects after their hospital admission diagnosed with CAD but before onset of any treatment and medication.

5 mL fasting blood samples were drawn from all subjects under aseptic precautions. After collection of blood samples, plasma of each sample was separated. Total cholesterol (TC) was determined with the help of diagnostic kit (DiaSys, USA) following the method mentioned by Richmond [10]. High density lipoprotein cholesterol (HDL-c) was determined using kit (Randox laboratories, USA) according to procedure of Lopes-Virella et al. [11]. Triglyceride (TG) was estimated using diagnostic kit (DiaSys, USA) according to Koditschek and Umbreit [12]. The concentration of low density lipoprotein-cholesterol (LDL-c) was determined by equation developed by Friedewald et al. [13]. Then fasting blood glucose level of each subject was measured following the clinical guidelines of American Diabetes Association [14]. For determination of post prandial (PP) blood glucose level each subject was asked to drink 75 g anhydrous glucose in 200 ml of water and a second blood sample was collected after 2 h. Hemoglobin (Hb) was estimated using Hemoglobin Colorimetric Assay Kit (Cayman chemical, USA). Serum creatine phosphokinase (CPK) and cardiac-specific CPK (MB isoenzyme) were determined using Creatine Kinase (CK) Activity Colorimetric Assay Kit (BioVision, USA) and Creatine Kinase (CPK-MB) Enzyme Immuno Assay Test Kit (OxisResearch, USA) respectively. Plasma homocysteine (Hcy) was determined followed the method mentioned by Frick et al. [15], using high-performance liquid chromatography (Waters, USA).

All data obtained were compared with the recommended normal value as per guidelines of Longo et al. [16], American Diabetes Association (ADA) [17], and American Heart Association (AHA) [18].

Daily dietary consumption of fat, carbohydrate as well as the major nutrients of interest of the present study directly related to the development of CAD i.e., vitamin B_6, vitamin B_{12} and folic acid were assessed by self reported questionnaire method taking account of the dietary habit of the subject for a period of five years just before diagnosis of the disease and obtained data were compared with the recommended values of daily dietary intake proposed by Indian Council of Medical Research (ICMR) [19].

2.3 Statistical Analysis

All the values in the present study are expressed as Mean ± SD (standard deviation). Pearson product moment correlation coefficient (r) and standard error of r (Sr) were determined. Data were analyzed by student t test using the software Microsoft Excel-2007. P values < 0.05 were considered as significant.

III. Results

3.1 Assessment of the status of the different risk factors

Present study showed that BMI, SBP, DBP, LDL-c, triglyceride, total cholesterol, fasting and post prandial blood glucose level, homocysteine, CPK and CPK-MB enzyme levels were higher while HDL-c, LVEF and hemoglobin levels were lower than recommended normal values in Group I and Group II (Table 1). We also observed that carbohydrate, vitamin B_6, vitamin B_{12} and folic acid consumptions were lower while fat consumption was higher in Group I and Group II than normal value of recommendation (Table 2). Comparison of different risk factors between Group I and Group II are also made (Table 1 and 2).

3.2 Analysis of correlation between dietary consumption of the vitamins and risk factors

We found that there was a significant negative correlation between intake of vitamin B_6 and BMI ($p < 0.0005$ for Group I and $p < 0.005$ for Group II) (Table 3) while there was no significant correlation between vitamin B_6 and the other risk factors (Table 3). On the other hand, it was observed that vitamin B_{12} had

significant ($p < 0.01$ and $p < 0.0005$ for Group I and Group II respectively) positive correlation with HDL-c level (Table 3). In Group I and Group II it was further observed that LDL-c and TC level were significantly negatively correlated with vitamin B_{12} ($p < 0.005$ and $p < 0.01$ for Group I and $p < 0.0005$ and $p < 0.01$ for Group II respectively) (Table 3). Moreover, in Group I and Group II hemoglobin level was found to be significantly positively correlated ($p < 0.01$ and $p < 0.005$ respectively) with vitamin B_{12} while both group had significant negative correlation with homocysteine level ($p < 0.0005$ and $p < 0.0005$ respectively). Finally, it was found that in case of both Group I and Group II, BMI was significantly ($p < 0.0005$ and $p < 0.0005$ respectively) negatively correlated with folic acid (Table 3). Moreover, SBP and DBP of both Group I and Group II showed a significant negative correlation with folic acid ($p < 0.005$ and $p < 0.0005$ for Group I and $p < 0.0005$ and $p < 0.005$ Group II respectively) (Table 3). Like vitamin B_{12} folic acid was also significantly positively correlated with HDL-c level in the subjects of Group I and Group II ($p < 0.0005$ and $p < 0.0005$ respectively) (Table 3). In Group I and Group II it was further observed that LDL-c and TC level were significantly negatively correlated with folic acid ($p < 0.0005$ and $p < 0.005$ for Group I and $p < 0.01$ and $p < 0.01$ for Group II respectively) (Table 3). Similarly, it was also found that blood sugar level (fasting) of both Group I and Group II had significant ($p < 0.005$ and $p < 0.0005$ respectively) negative correlation with folic acid (Table 3). Hemoglobin level of Group I and Group II was found to be positively correlated ($p < 0.0005$ and $p < 0.01$ respectively) with folic acid, whereas homocysteine levels of both groups were found to be significantly negatively correlated with folic acid ($p < 0.0005$ and $p < 0.0005$ respectively) (Table 3).

IV. Discussion

Vitamin B_6, vitamin B_{12} and folic acid have been reported to play an important role in reducing risk factor for the development of CAD [20]. In addition to this vitamin B_6, vitamin B_{12} and folic acid were reported to reduce homocysteine level which confers independent risk for CAD through damaging the endothelial epithelium, smooth muscle proliferation and enhancing lipid peroxidation [3]. Robinson et al. [21] demonstrated that low level of folic acid and vitamin B_6, vitamin B_{12} are often seen in patients with atherosclerosis. Moreover, deficiencies of these vitamins were also reported earlier to be prevalent in Indian population leading to the development of CAD [20].

In the present study patients with CAD of both genetic and non-genetic background, were found to consume low vitamin B_6, vitamin B_{12} and folic acid (Table 2) than the recommended normal values and also found to have high level of homocysteine (Table 1). Thus, low consumption of these vitamins may be responsible for high level of homocysteine which may be the underlying reason for development of CAD in both groups in the present study (Table 1 and 2). Additionally it was also observed in the present study that consumption of vitamin B_6, vitamin B_{12} and folic acid (Table 2) in patients with genetic background was significantly ($p < 0.0005$, $p < 0.0005$ and $p < 0.025$ respectively) lower than the patients with non-genetic background and these differences in consumption of the three vitamins (Table 2) may be correlated with observed significant ($p < 0.025$) higher level of homocysteine in patients with genetic background (Table 1). Several studies showed that adequate amount of folate and vitamin B_{12} has an important role in erythropoiesis and deficiency of either vitamin causes megaloblastic anemia [22]. In addition to this, Chonchol and Nielson [23] reported that low hemoglobin is related to the increased risk for CAD and also with the new events for coronary attack. Our results also reveal that both groups of patients had low level of hemoglobin (Table 1). So, it can be said that deficiency of vitamin B_{12} and folic acid may be the cause of low level of hemoglobin in both groups and differences in consumption of these vitamins may be the reason for significant ($p < 0.01$) differences in hemoglobin level between the two groups (Table 1 and 2).

Several studies well documented that family history, age, gender, smoking, lack of physical activity, hypertension, obesity, diabetes, hyperlipidemia are associated with CAD [4, 5]. James [2] reported that among South Indian population irrespective of gender, diabetes mellitus and dyslipidemia are the major risk factor for CAD. Deb and Dasgupta [24] reported that in Kolkata prevalence of hypertension and diabetes in 25-59 yrs are higher than in North India. Like the previous reports, we also found overweight, hypertension and hyperlipidemia to be prevalent as CAD risk factors in patients from both background (Table 1) in this study.

On the other hand, when we compared the mean values of different risk factors between the two groups, it was observed that patients from genetic background had significantly higher level of SBP ($p < 0.0005$), DBP ($p < 0.005$), LDL-c ($p < 0.025$), TG ($p < 0.0005$), TC ($p < 0.005$) and significantly ($p < 0.025$) lower level of HDL-c than the subjects from non-genetic background (Table 1) indicating patients from genetic background were more susceptible to CAD than the patients from non-genetic background.

Most interestingly in the present study we also found that subjects from genetic background were diabetic and had glucose intolerance while diabetes mellitus as well as glucose intolerance was not found in the subjects from non-genetic background. Regarding diabetes mellitus, results of our study (Table 1) suggest that patients from genetic background were at very high risk for CAD. According to recommendation of American Diabetes Association [17] and American Heart Association [18], diabetic patient should maintain SBP < 130

mmHg, DBP < 90 mmHg, blood HDL-c > 40 mg/dl, LDL-c < 70 mg/dl, TC < 150 mg/dl, TG < 100 mg/dl. Besides, it was reported that reduced LVEF, increased level of CPK and CPK-MB enzyme are associated with acute myocardial infarction and development of CAD [25, 6]. In this study subjects from both backgrounds were also found to have low LVEF but high levels of CPK and CPK-MB enzyme (Table 1) than recommended normal values which indicates occurrence of myocardial infarction in the study subjects.

On the other hand, suggestion was there that lack of physical activity increases BMI and also has a positive association with obesity which increases the risk of elevation of blood sugar level, hypertension, and hypercholesterolemia [26, 27]. Additionally, it was also reported that in India lack of physical activity, cholesterol-rich diet are the major reasons for inducing CAD [27, 5]. In the present study, during subject selection we found all the patients from both background to have sedentary life style (data not shown) according to their daily energy expenditure [10]. Moreover, we also observed from the records of dietary intake of the patients from both groups that they used to intake high level of fat and low level of carbohydrate (Table 2) in their diet before diagnosis of CAD. Hence, sedentary life style as well as high fat consumption may be responsible for overweight as well as on set of CAD in the study subjects [26, 27].

On the other hand, Mierzecki [28] has shown that folic acid supplementation can reduce TC, LDL-c, homocysteine level and BMI in both male and female with atherosclerosis risk factors. Papandreou et al. [29] has also reported that there was negative association between intake of folic acid and homocysteine level while homocysteine level has positive association with BMI and blood glucose level. Moreover, supplementation of vitamin B_6 and vitamin B_{12} in combination of folic acid has been also reported to decrease the level of risk factors of CAD in Indian population [26]. On further analysis of the correlation between the vitamins (B_6, B_{12} and folic acid) (Table 3) and different risk factors for CAD it was also observed that vitamin B_{12} and folic acid had significant and strong correlation with the major risk factors for CAD while vitamin B_6 had no significant association with these different risk factors in the subjects from both background. Gilbert et al. [30] also reported that B_6 has no effective role in reducing cardiac risk factors. In addition to this, Robinson et al. [21] reported that low vitamin B_6 is often associated with high homocysteine level but risk for CAD is not mediated through the high homocysteine level in case of vitamin B_6.

V. Tables

Table -1: Comparison of risk factors for CAD between Group I and Group II.

Risk factors	Group		RDA[a]	P[b]
	Group I (n = 85)	Group II (n = 87)		
BMI, Kg/m^2	26.75 ± 3.52	26.67 ± 3.34	18.5-22.9	NS
SBP, mm/Hg	166 ± 15.89	158 ± 15.35	140	< 0.0005
DBP, mm/Hg	98.4 ± 10.35	94 ± 10.21	90	< 0.005
HDL-c, mg/dL	33.25 ± 9.25	36.67 ± 10.25	40-80	< 0.025
LDL-c, mg/dL	135.45 ± 10.75	131.8 ± 9.89	70-100	< 0.025
TG, mg/dL	181.92 ± 31.11	164.09 ± 29.14	150	< 0.0005
TC, mg/dL	210.45 ± 17.25	203.15 ± 15.35	140-200	< 0.005
Blood sugar (Fasting), mg/dL	145.7 ± 29.13	103.68 ± 27.73	65-115	< 0.0005
PP blood glucose, mg /dL	210.72 ± 95.83	129.2 ± 18.51	140-200	< 0.0005
LVEF, %	41.89 ± 12.5	42.09 ± 9.43	<55	NS
Haemoglobin, gm/dL	11.9 ± 1.6	12.6 ± 2	13-17	< 0.01
CPK, IU/L	297.19 ± 73.17	296.95 ± 71.1	51-294	NS
CPK-MB, µg/ml	19.96 ± 12.21	20.03 ± 17.21	0-5.5	NS
Hcy, µmol/L	22.5 ± 3.89	21.3 ± 3.67	5-15	< 0.025

[a] RDA: Recommended Dietary Allowances.

[b] Values are expressed as MEAN± standard deviation. Level of significance: Student's t-test, was performed to determine whether there was any significant difference in the mean values of CAD risk factors between Group I

(n=85) and Group II (n=87). $p < 0.0005$, significant; $p < 0.005$, significant; $p < 0.01$, significant; $p < 0.025$, significant; NS, not significant.

Table -2: Comparison of nutritional status between Group I and Group II.

Dietary consumption	Group		RDA[a]	P[b]
	Group I (n = 85)	Group II (n = 87)		
Carbohydrate, gm/day	209.5 ± 24.72	231 ± 29.27	290	< 0.0005
Fat, gm/day	66.5 ± 8.14	73 ± 9.13	25-40	< 0.0005
vitamin B_6, mg/day	1.28 ± 0.24	1.38 ± 0.25	2	< 0.0005
vitamin B_{12}, µg/day	0.405 ± 0.17	0.58 ± 0.24	1	< 0.0005
folic acid, µg/day	54.31 ± 7.81	57.3 ± 9.7	200	< 0.025

[a] RDA: Recommended Dietary Allowances.
[b] Values are expressed as MEAN± standard deviation. Level of significance: Student's t-test, was performed to determine whether there was any significant difference in the mean values of dietary consumption of carbohydrate, fat and vitamins (vitamin B_6, vitamin B_{12} and folic acid) between Group I (n=85) and Group II (n=87). $p < 0.0005$, significant; $p < 0.005$, significant; $p < 0.01$, significant; $p < 0.025$, significant; NS, not significant.

Table -3: Correlation between dietary consumption of the vitamins (B_6, B_{12} and folic acid) and risk factors of CAD.

Variable	Vitamin B6 Group		Vitamin B12 Group		Folic acid Group	
	Group I (n = 85)	Group II (n = 87)	Group I (n = 85)	Group II (n = 87)	Group I (n = 85)	Group II (n = 87)
BMI, Kg/m2	-0.629	-0.315	0.123	0.074	-0.661	-0.415
p	< 0.0005	< 0.005	NS	NS	< 0.0005	< 0.0005
SBP, mm/Hg	-0.168	-0.108	-0.015	-0.075	-0.313	-0.439
p	NS	NS	NS	NS	< 0.005	< 0.0005
DBP, mm/Hg	0.103	-0. 112	-0.092	-0.109	-0.430	-0.312
p	NS	NS	NS	NS	< 0.0005	< 0.005
HDL-c, mg/dL	-0.137	0.002	0.265	0.513	0.557	0.513
p	NS	NS	< 0.01	< 0.0005	< 0.0005	< 0.0005
LDL-c, mg/dL	0.032	0.127	-0.293	-0.629	-0.473	-0.269
p	NS	NS	< 0.005	< 0.0005	< 0.0005	< 0.01
TG, mg/dL	0.053	0.054	0.042	-0.013	-0.040	-0.081
p	NS	NS	NS	NS	NS	NS
TC, mg/dL	-0.126	-0.079	-0.263	-0.259	-0.297	-0.265
p	NS	NS	< 0.01	< 0.01	< 0.005	< 0.01
Blood sugar level (Fasting), mg/dL	0.115	0.051	-0.054	-0.122	-0.287	-0.473
p	NS	NS	NS	NS	< 0.005	< 0.0005
LVEF, %	0.122	0.066	0.168	0.131	0.065	-0.103
p	NS	NS	NS	NS	NS	NS
Hb, gm/dL	0.119	0.043	0.270	0.289	0.443	0.258
p	NS	NS	< 0.01	< 0.005	< 0.0005	< 0.01
CPK, IU/L	0.068	0.027	-0.118	-0.127	0.048	0.003
p	NS	NS	NS	NS	NS	NS
CPK-MB, µg/mL	-0.111	0.042	0.042	-0.030	-0.143	-0.011
p	NS	NS	NS	NS	NS	NS
Hcy, µmol/L	-0.046	-0.032	-0.423	-0.446	-0.493	-0.459
p	NS	NS	< 0.0005	< 0.0005	< 0.0005	< 0.0005

Pearson product moment r values are given. Level of significance: Student's t-test, was done to determine whether there was any significant correlation between the mean values of dietary vitamins (B_6, B_{12} and folic acid) and CAD risk factors of Group I and Group II. $p < 0.0005$, significant; $p < 0.005$, significant; $p < 0.01$, significant; $p < 0.025$, significant; NS, not significant.

VI. Conclusion

Finally data obtained from the present study reveal that sedentary life style, consumption of high fat along with low consumption of vitamin B_6, vitamin B_{12} and folic acid as well as high plasma homocysteine level might be underlying reasons for CAD development in the present subject groups with genetic and non-genetic

backgrounds for CAD from Kolkata, India. Significantly higher prevalence of diabetes mellitus in the patients with genetic background elevated the chance of the disease causation in them.

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References

[1]. R. Lozano, Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010, Lancet, 380, 2012, 2095–2128.
[2]. C. James, Risk factors for coronary artery diseases: A study among patients with ischemic heart disease in Kerala, Heart India, 1, 2013, 7–11.
[3]. K.M. Fairfield and R.H. Fletcher, Vitamins for chronic disease prevention in adults: scientific review, JAMA, 287, 2002, 3116–3226.
[4]. R. Gupta and V.P. Gupta, Meta–analysis of coronary heart disease prevalence in India, Indian Heart Journal, 48, 1996, 241–245.
[5]. A.J. Lusis, A.M. Fogelman and G.C. Fonarow, Genetic basis of atherosclerosis: Part I: New genes and pathways, Circulation, 110, 2004, 1868–1873.
[6]. G.M. Galen, The enzyme diagnosis of myocardial infarction, Human Pathology, 6, 1975, 141.
[7]. J.A. Finegold, P. Asaria and D.P. Francis, Mortality from ischaemic heart disease by country, region, and age: statistics from World Health Organisation and United Nations, International journal of cardiology, 168, 2012, 934–945.
[8]. Y. Venkata Ramana, M.V.L. Surya Kumari, S. Sudhakar Rao and N. Balakrishna, Variations in basal metabolic rate with incremental training load in athletes, Journal of Exercise Physiology Online, 7, 2004, 26–33.
[9]. World Health Organization Expert Committee, Measuring obesity–classification and description of anthropometric data, Copennhagen, Regional office for Europe: WHO, 1989.
[10]. W. Richmond, Preparation and properties of a cholesterol oxidase from Nocardia sp and its application to the enzymatic assay of total cholesterol in serum, Clinical Chemistry, 19, 1973, 1350–1356.
[11]. M.F. Lopes–Virella, P. Stone, S. Ellis and J.A. Colwell, Cholesterol determination in high–density lipoproteins separated by three different methods, Clinical Chemistry, 23, 1977, 882–884.
[12]. L.K. Koditschek and W.W. Umbriet, Alpha–glycerophosphate oxidase in Streptococcus facium F24, Journal of Bacteriology, 98, 1969, 1063–1068.
[13]. W.T. Friedewald, R.I. Levy and D.S. Fredrickson, Estimation of the concentration of low–density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clinical Chemistry, 18, 1972, 499–502.
[14]. American Diabetes Association, Standards of medical care in diabetes–2007, Diabetes Care, 30, 2007, S4–S41.
[15]. B. Frick, K. Schrocksnadel , G. Neurauter, B. Wirleitner, E. Artner-Dworzak and D. Fuchs, Rapid measurement of total plasma homocysteine by HPLC, Clinical Chemistry Acta, 331, 2003, 19–3.
[16]. D.L. Longo, A.S. Fauci, D.L. Kasper, S.L. Hauser, J.L. Jameson and J. Loscalo, Harrison's principles of internal medicine (18th ed, NY: McGraw–Hill Professional, 2012, 3590–3591).
[17]. American Diabetes Association, Standards of medical care in diabetes, Diabetes Care, 35, 2012, S11–S63.
[18]. American Heart Association, Cholesterol abnormalities and diabetes, 2013.
[19]. Indian Council of Medical Research (ICMR), Nutrient requirement and recommended dietary allowances for Indians: A report of the Expert Group of the Indian Council of Medical Research, NIN, Hyderabad: ICMR, 2010, 1–51.
[20]. S.K. Gupta, J. Kotwal, A. Kotwal, A. Dhall and S. Garg, Role of homocysteine and MTHFR C677T gene polymorphism as risk factors for coronary artery disease in young Indians, Indian Journal of Medical Research, 135, 2012, 506–512.
[21]. K. Robinson, K. Arheart, H. Refsum, L. Brattstrom, G. Boers, P. Ueland, P. Rubba, R. Palma-Reis, R. Meleady, L. Daly, J. Witteman and I. Graham, Low circulating folate and vitamin B6 concentrations risk factors for stroke, peripheral vascular disease, and coronary artery disease, Circulation, 97, 1998, 437–443.
[22]. M.J. Koury, D.W. Horne, Z.A. Brown, J.A. Pietenpol, B.C Blount, B.N. Ames, R. Hard and S.T. Koury, Apoptosis of late stage erythroblasts in megaloblastic anemia: association with DNA damage and macrocyte production, Blood, 89, 1997, 4617–4623.
[23]. M. Chonchol and C. Nielson, Hemoglobin levels and coronary artery disease, American Heart Journal, 155, 2008, 494-498.
[24]. S. Deb and A. Dasgupta, A study on risk factors of cardiovascular diseases in an urban health center of Kolkata, Indian Journal of Community Medicine, 33, 2008, 271–275.
[25]. R.A. Schulze, H.W. Strauss and B. Pitt, Sudden death in the year following myocardial infarction. Relation to ventricular premature contractions in the late hospital phase and left ventricular ejection fraction, American Journal of Medicine, 62, 1977, 192–198.
[26]. L.H. Mortensen, I.C. Siegler, J.C. Barefoot, M. Gronbaek and T.I. Sorensen, Prospective associations between sedentary lifestyle and BMI in midlife, Obesity, 14, 2006 1462–1471.
[27]. P.P. Joshi, Why is coronary heart diseases increasing in Indians? Cardiovascular risk factors in the Indian scenario, South Asian Journal of Prevention Cardiology, 2003, 7:4.
[28]. A. Mierzecki, Association between low–dose folic acid supplementation and blood lipids concentrations in male and female subjects with atherosclerosis risk factors, Medical Science Monit, 19, 2013, 733–739.
[29]. D. Papandreou, P. Malindretos, M. Arvanitidou, A. Makedou and I. Rousso, Oral supplementation of folic acid for two months reduces total serum homocysteine levels in hyperhomocysteinemic Greek children, Hippokratia, 14, 2010, 105–108.
[30]. S.O. Gilbert, A.A.B. Shirley and G.M. Arno, Preventing coronary heart disease: B Vitamins and homocysteine, Circulation, 97, 1998, 421–424.