



Original Research Article

Oxidative stress in individuals on different dietary patterns- A comparative study

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ABSTRACT

Introduction: The major chronic disorders such as cancer, cardiac ailments and diabetes are global health burden and the dietary habits playing a significant role in setting these among other factors. There is an assumption that non-vegetarian diet produces more free radicals (FR) when compared to vegetarian food. However recent studies showed an equal contribution of FR injury in vegetarians too. The assessment of the FR markers may provide an analytical report in correlation with different dietary pattern.

Materials and Methods: In this comparative study, the individuals (age group of 15 to 50 years) with their dietary habits of vegetarian (n=60) and of non-vegetarian diet (n=60), were included. The serum was analysed for the parameters, fasting blood sugar (FBS), Lipid Profile, MDA, IMA and oxidised LDL (oxLDL). The quantitative variables were analysed by appropriate statistical methods and the p value<0.05 is considered statistically significant.

Results: The comparative values are expressed as mean \pm SD for vegetarians and non-vegetarian groups and it is evident that the FBS and lipid profile and oxLDL were statistically non-significant. Malondialdehyde (MDA) and Ischemia modified albumin (IMA) are significantly raised in non-vegetarian group in comparison to the vegetarian group (p<0.05).

Conclusion: Based upon the dietary patterns in the healthy individuals, the oxidative stress markers i.e. MDA, IMA are showed conspicuous difference and oxLDL is not significant. The ratio of IMA/MDA in non vegetarians has been considered for the intensity of the oxidative stress beyond the specified ratio (18.44).

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1. Introduction

In the present day scenario, the close relation between the life style related diseases and oxidative stress is well known. The consumption of vegetables and low fat diet reduces the risk of several health disorders. However, this is again associated with the demographic and socioeconomic factors like age, education, religion, income, marital status etc. The life style factors such as alcohol consumption, smoking and physical activity also influences the risk for diseases. Based upon the dietary pattern, the non vegetarian diet is defined as consumption of meat (red meat, poultry at least once

per month) and the total meat and fish consumption for more than one time in a week. Such people are called non vegetarians. Whereas, those on food totally devoid of meat and dairy products are considered as strict vegetarians.¹ Food is usually of plant or animal origin and contains essential nutrients such as carbohydrates, fats, proteins, vitamins and minerals. Vegetarians are observed to have reduced risk of many chronic diseases and recent studies have shown that compared to vegetarian diet. The non-vegetarian food produces more free radicals with increased lipid peroxidation and low antioxidant status.²

The FR are the uncharged molecule (typically highly reactive and short-lived) having an unpaired valence

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electron in their outermost orbit. The FR interacts with the molecules within the cells causing damage (or oxidative stress) and also the adjacent cells face the insult. Oxidative stress occurs when an over load of FR that cannot be destroyed accumulate in the body i.e., an imbalance occurs between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or repair the damage caused by them.³

Clinical and experimental evidence proves that FR plays a major role in many physiological and pathological conditions. The wide spread application of FR measurement has increased the awareness and assessment of functional impairment. In recent years, oxidative stress due to reactive oxygen species (ROS) is implicated in the pathogenesis of many of diseases like cancer, cataract, diabetes mellitus, rheumatoid arthritis, atherosclerosis, viral and autoimmune diseases.⁴ The FR and other reactive oxygen species (ROS) can also be derived from external sources such as exposure to X-rays, Ozone, cigarette smoking, air pollutants, and industrial chemicals etc.⁵

1.1. Malondialdehyde (MDA)

It is a highly reactive three carbon dialdehyde produced as a by-product of peroxidation of polyunsaturated fatty acids. MDA readily combines with several functional groups on molecules including proteins, lipoproteins and DNA causing damage. The toxicity of MDA may results in mutagenicity and cancer.⁶

1.2. Ischemia Modified Albumin (IMA)

Human serum albumin (HSA) is the major constituent (60%) of plasma proteins. Albumin is more prone for modification by both enzymatic and non-enzymatic methods. It maintains the plasma oncotic pressure, solubilisation, transportation and buffering actions. IMA, an altered form of albumin, was first identified in the early 1990s. The formation of IMA is directly influenced by reactive oxygen species (ROS) which is generated during oxidative stress including myocardial ischemia. The NH₂ terminal of albumin tends to bind metal ions such as cobalt, copper, nickel, aluminium and cadmium. The strong affinity of albumin to metal ions at the binding site is due to the three NH₂-terminal amino acid residues Asp-Ala-His along with lysine residue designated as Lys4.⁷

The generation of free radicals (or ROS) alter the NH₂-terminus of albumin which loses its ability to bind with cobalt, copper and other metal ions which is the basis of the albumin cobalt binding test (ACB test) for IMA. Thus, the increased amounts of IMA result in less cobalt binding. The more residual unbound cobalt is available for complex with a chromogen (dithiothreitol) that can be measured photometrically. Studies reported that, IMA is not only

specific for cardiac ischemia as the levels are also increased in all FR associated disorders including cirrhosis of the liver, advanced cancers, brain stroke, end stage renal disease (ESRD) and many acute infections.^{8,9}

1.3. Oxidised LDL

It is produced when low density lipoprotein (LDL) particles react with FR and this association shows a great impact in the form of numerous life style disorders.¹⁰

2. Materials and Methods

2.1. Selection of participants

The present study was carried out at Maharajah's Institute of Medical Sciences (MIMS), Nellimarla with the approval of institutional ethical committee. The Healthy individuals of both the genders with an age group ranging from 15-50 and with their different dietary habits i.e., vegetarian and non-vegetarian diet, were included. The vegetarian group comprises of 60 individuals and non-vegetarian group consists of 60 individuals. Informed consent was obtained from all the participants.

2.2. Inclusion criteria and exclusion criteria

The healthy participants who fulfilled the criteria of vegetarian and non vegetarian dietary patterns as per WHO guidelines were included. The participants with smoking, obesity, diabetes mellitus, hypertension, dyslipidemia, infectious diseases, inflammatory conditions and cardiac ailments were excluded from the study. The enrolment of the participants in this study was based on their dietary intake and was assessed by a validated questionnaire.

2.3. Sample collection

In this analytical cross sectional study, with the approval of institutional ethical committee and obtaining the informed consent from the participants, a detailed clinical examination was performed upon them with reference to age, sex, life style and family history. The participants were patient attendants, students, healthy volunteer and people who came for routine health check-up. 5ml of plain blood sample was collected, separated and the serum samples were preserved at -40°C for batch wise analysis. The serum was analysed in the laboratory for fasting blood sugar (FBS), Lipid Profile, Malondialdehyde (MDA), Ischemia modified albumin (IMA) and oxidised LDL as per the guidelines provided for the respective tests.

2.4. Statistical analysis

The quantitative variables and the descriptive measures of the subjects were expressed as percentages. Normally distributed data variables were presented in Mean and

Standard deviation. The Student independent sample “t” test is used for mean comparison between two groups and for all statistical analysis, the $p < 0.05$ is considered as statistically significant.

2.5. Methods: Estimation of biochemical parameters

Fasting blood sugar (FBS): Estimated by glucose oxidase and peroxidase method.¹¹ Lipid Profile: The serum total cholesterol was estimated by cholesterol oxidase and peroxidase method (CHOD-POD). Hydrogen peroxide is formed during the cholesterol oxidation and in turn this oxidises the chromogen to produce pink colour.¹² Serum triglyceride was estimated by Trinder method (dynamic extended stability with lipid clearing agent).¹³ The HDL cholesterol was analysed by Phosphotungstic Acid Method.¹⁴ Chylomicrons, LDL and VLDL are precipitated from serum by phosphotungstate. The HDL cholesterol remains unaffected in the supernatant and is estimated with enzymatic cholesterol method. The serum LDL cholesterol and serum VLDL cholesterol were measured by indirect method in accordance with Friedewald formula. All these parameters were analysed on Transasia Erba fully automated analyser.

Estimation of IMA: Ischemia modified albumin (IMA) is measured by albumin cobalt binding assay. In which, a known amount of cobalt is added to serum sample and then unbound cobalt is measured by the intensity of coloured complex formed after reacting with dithiothreitol by using colorimeter at 470nm. The values expressed in U/mL extrapolated from standard graph and one IMA unit is defined as ‘gm of free cobalt’.¹⁵

Estimation of MDA: Assayed with Thiobarbituric acid by Keisatoh method.¹⁶ The lipid peroxide content is estimated as MDA equivalent of TBA assay values which measures total MDA (free and unbound)

Estimation of oxidised LDL: ELISA method (double sandwich method) by using Elico ELISA reader, in which an antigen is caught between two antibodies. The standards and samples were added to the micro ELISA plate for the reaction with specific antibody. This is followed by the addition of Human OxLDL and Avidin-Horseradish Peroxidase (HRP) conjugate for specific antibody and incubated. Free components are washed away and only those wells that contain Human OxLDL, biotinylated detection antibody and Avidin-HRP conjugate that appeared in blue colour. After adding the stop solution the colour turns to yellow and measured at $450 \text{ nm} \pm 2 \text{ nm}$.¹⁷

3. Results

The present study was undertaken to observe the free radical status in apparently normal healthy individuals with different dietary patterns i.e. vegetarian and non-vegetarian food types. Among the total 120 healthy subjects, i.e.

vegetarians (n=60) and non vegetarians (n=60), the males comprise of 57% and 70% and females were 43% and 30% for vegetarian and non-vegetarian groups respectively (Table 1). The characteristics of the basic parameters were shown for both the groups in Table II. The mean comparative values are expressed as mean \pm SD and it is evident that the FBS and lipid profile (TAG, total cholesterol, HDL, LDL and VLDL) were statistically non significant. The oxidative stress markers i.e. MDA, IMA and oxLDL in non-vegetarians are higher when compared to vegetarians. Here, with oxLDL, the elevation is not significant statistically whereas the other two markers i.e. MDA and IMA showed conspicuous difference...p value (Table III). The Table IV demonstrated the ratio of the stress markers IMA/MDA. The ratio of these parameters (18.44) has been occurred in this study is considered for the intensity of the oxidative stress of an individual. The cutoff beyond this specified ratio indicates the risk for the future complications that are generated by free radical injury.

Table 1: Distribution of groups – gender wise

Group	Male (%)	Female (%)
Vegetarians	34 (57)	26 (43)
Non Vegetarians	42 (70)	18 (30)

The gender distribution is 57% and 70% of males for vegetarian and non-vegetarian groups respectively. The females contributed 43% and 30% for vegetarian and non-vegetarian groups respectively.

Comparative values are expressed as mean \pm SD for vegetarians and non-vegetarian groups and it is evident that the FBS and lipid profile (TAG, total cholesterol, HDL, LDL and VLDL) were statistically non significant.

From the Table 3 it is evident that serum MDA and IMA is significantly raised in non-vegetarian groups in comparison to vegetarians ($p < 0.05$), whereas oxLDL is statistically non significant.

4. Discussion

The current study demonstrated that, in comparison to non-vegetarians, the vegetarians showed relatively lower levels of FBS and lipid profile except for VLDL. However, the difference in decrease is statistically not significant. From the table I, it is evident that, the male participants are more when compared to female in both the groups. During this study period, it was hard to find the strict vegetarians to enrol in the study and several studies suggesting that the consumption of non-vegetarian products is rampantly increasing globally.¹

In comparison to vegetarians, the non-vegetarians showed a slight elevation in the FBS levels but statistically not significant and they are in the normal range. The present study is in corroboration with the studies of Marco et al, 2013 and Demosthenas et al, 2005. The consumption of

Table 2: A comparative study of Fasting blood sugar, lipid profile in vegetarians and non-vegetarian dietary pattern groups

Parameters mg/dL	vegetarians* (Mean \pm SD)	non vegetarians (Mean \pm SD)	p value
FBS	79.86 \pm 10.46	86.49 \pm 9.87	NS
TAG	72.96 \pm 37.89	91.11 \pm 53.67	
TC	161.56 \pm 40.71	168.21 \pm 33.95	
HDL	36.81 \pm 6.19	40.83 \pm 16.45	
LDL	106.48 \pm 40.04	117.76 \pm 35.14	
VLDL	20.13 \pm 8.25	18.19 \pm 10.71	

FBS = fasting blood sugar, TAG = triacylglyceride, TC = total cholesterol, HDL= high density lipoprotein, LDL= low density lipoprotein, VLDL= very low density lipoprotein, N.S = non significant.

Table 3: A comparative study of Malondialdehyde, ischemia modified albumin and oxidized LDL in the different dietary pattern groups

Parameters mg/dL	Vegetarians (Mean \pm SD)	non vegetarians (Mean \pm SD)	p value
MDA (mmol/mL)	4.09 \pm 1.00	6.30 \pm 2.49	<0.05
IMA (U/mL)	58 \pm 3.86	90.65 \pm 17.35	<0.05
oxLDL (pg/mL)	6.51 \pm 4.97	7.15 \pm 3.84	NS

MDA= malondialdehyde, IMA= ischemia modified albumin, oxLDL = oxidized low density lipoprotein

Table 4: Ratio of serummalondialdehyde and ischemia modified albumin in the individuals of different dietary patterns

Test	Vegetarians	Non Vegetarians	P value
IMA/MDA	15.36 \pm 6.91	18.44 \pm 0.034	NS

NS = non significant

high intake of fat and meat may induce hyper insulinemia and insulin resistance in otherwise healthy individuals. Such a situation is directed towards metabolic syndrome and hence other associated clinical complications. This entire mechanism is directly influenced by the reactive oxygen species (ROS).^{18,19}

In case of Lipid profile, the levels are slightly elevated in non-vegetarian group when compared to the vegetarian group except for VLDL. However the difference is not statistically significant. LDL is considered as one of the most potent risk factor for ischemic diseases. Studies reported that, the individuals with mixed diet especially high fat and meat products registered more serum cholesterol levels. This is due to the obvious contribution of food source.²⁰ HDL is considered as good cholesterol and interestingly the present study registered elevated plasma HDL levels in non-vegetarians than vegetarians. But, in contrast to other studies, the current study showed increased VLDL levels in vegetarian groups.²¹

According to the findings in the table-III, the free radical markers i.e. MDA, IMA and oxLDL showed marked elevation in non-vegetarians in comparison to vegetarians. The difference in MDA and IMA levels is statistically significant and the ox LDL is insignificant. The rise of all these parameters can be accounted for free radical generation. In case of MDA, our findings are in corroboration with the study of Parvin Mirmiran et al, 2018. Studies stated that irrespective of being a vegetarian or a non-vegetarian, the high intake of fast foods, processed foods, meat etc aggravates the oxidative stress.²² The oxidative stress is higher in sedentary, stressful life and with

bad eating habits such as consumption of high calorogenic food and meat. All these result in accumulation of FR which oxidise the LDL to oxLDL, an atherogenic factor.²³ The consumption of healthy diet, rich in vegetables, fruits and restricted dietary fat results in the depletion of cholesterol, triglycerides and oxLDL.²⁴

Another study by Miller E R et al, 1998 revealed that the consumption of vegetables and fruits can reduce the rate of lipid peroxidation.²⁵ The Free radicals that are generated in the body result in several disorders including ischemia. The serum albumin is converted to ischemia modified albumin (IMA) because of the FR effect. IMA can be used as a standard marker along with troponins if coronary ischemia is the suspicion. Studies showed that increased IMA levels are attributed to FR either directly or indirectly.²⁶

The individual oxidative stress markers may vary with different factors. Hence the present study adopts combination in establishing the ratio of potent markers of oxidative stress rather than the individual markers. The timely screening of these markers helps in understanding the status of the FR generation. This may be helpful to prevent further complications associated with it by instituting appropriate precautionary measures.

5. Conclusion

Compared to the oxidative stress parameters included in this study i.e. OxLDL, IMA and MDA, the oxLDL showed only a minor difference among vegetarians and non vegetarians in our study. However, there is no significant difference in the ratio of IMA/MDA and the value obtained in non

vegetarians taken as bench mark. The ratios higher than 18.44 is considered as intensity of the free radical injury. Thus the assessment of the FR markers or the ratio time to time in apparently healthy individuals gives an insight into future free radical associated disease. However, further studies among the general population to establish optimum cut off value are still required.

6. Source of Funding

MIMS organization.

7. Conflict of Interest

None.

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