ASSOCIATION OF DIETARY PATTERNS, WITH INFLAMMATORY BIOMARKERS, OXIDATIVE STRESS, AND ENDOTHELIAL FUNCTION

By

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In partial fulfillment of the requirements for the Master's degree in

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-Muskan M Page

List of Abbreviations

CVD: Cardiovascular disease

SFAs: Saturated fatty acids

PUFAs: Polyunsaturated fatty acids

DASH: Dietary approaches to stop hypertension

Cys: cysteine

CySS: disulfide cysteine

IL: interleukin

CNCDs: Chronic Non-Communicable Disease

EDH: Endothelium-dependent hyperpolarization

NO: Nitric oxide

ED: Endothelial dysfunction

GSH-px: Glutathione peroxidase

SOD: Superoxide dismutase

MDA: malondialdehyde

BH4: tetrahydrobiopterin

NADPH: Nicotinamide adenine dinucleotide phosphate

cGMP: Guanosine 3' ,5 monophosphate

NOS: Nitirc oxide synthase

eNOS: endothelial nitric oxide synthase

nNOS: neuronal nitric oxide synthase

iNOS: inducible nitric oxide synthase

CRP: C- reactive protein

Hs-CRP: High-sensitive C-reactive protein

ELISA: Enzyme Linked Immunosorbent Assay

RAP: resonant acoustic profiling

LDL: Low-density lipoprotein

PAI-1: Plasminogen activator inhibitor-1

AHA: American Heart Association

PCOS: Polycystic ovary syndrome

BMI: Body mass index

CAD: Coronary artery disease

ICAM: Intercellular adhesion molecule-1

CRF: Chronic renal failure

TBA: Thiobarbituric acid

TCA: Trichloroacetic acid

HCl: Hydrochloric acid

DW: Distilled water

OS: Oxidative stress

ROS: Reactive oxygen species

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ABSTRACT

Background: Diet influences inflammatory status, oxidant-antioxidant balance, and vascular health. Foods are consumed in different combinations, proportions, regularity, and quantity forming the basis of dietary patterns. There is a paucity of information on how different dietary patterns affect biological measures of inflammation, oxidation, and endothelial function.

Objectives: The present study aimed to assess the influence of vegetarian and non-vegetarian dietary patterns on markers of inflammation, oxidation, and endothelial function.

Methods: The present cross-sectional comparative study was conducted on 90 subjects divided into two groups: Group 1: Vegetarians (n=45); Group 2: Non-vegetarians (n=45). The anthropometric parameters like height (m), and weight (kg) was recorded. BMI (kg/m^2) was calculated. The physiological parameters like systolic blood pressure (SBP, mm Hg), diastolic blood pressure (DBP, mm Hg), and pulse rate (bpm) were recorded. Serum Hs-CRP (mg/l), Serum MDA (µmol/l), and Serum NO (mmol/l) were measured as markers of inflammation, oxidative stress, and endothelial function. Statistical analysis was done using SPSS (version 20).

Results: No significant differences were observed in the BMI and physiological parameters between vegetarians and non-vegetarians. Hs-CRP and Serum NO did not vary between the two groups. Serum MDA levels were significantly higher among vegetarians compared to non-vegetarians. A statistically significant correlation was observed between Serum MDA and age, which was not consistent in the two groups.

Conclusion: The results of the present study indicate an impact of vegetarian and non-vegetarian dietary patterns on oxidative stress with a better oxidative stress profile among non-vegetarians. The results of the present study warrant a detailed analysis of the adequacy of food groups, food variety, quality, and quantity of different food components. Also, there is a need to understand how food preferences change with age. Further studies are also needed to delineate the influence of lifestyle factors like a sedentary lifestyle, and levels of physical activity on markers of inflammation, oxidative stress, and endothelial functions in addition to dietary patterns.

Keywords: Dietary pattern, Vegetarian, Non-vegetarian, hs-CRP, Oxidative stress, Endothelial function.

INTRODUCTION

Inflammation is a key element of immunosurveillance and host defense, yet chronic low-grade inflammation is a pathological feature of a wide range of chronic conditions like metabolic syndrome, non-alcoholic fatty liver disease, hyperglycemia, and cardiac disease (CVD). It is likely that an unresolved inflammatory response may kickstart a number of diseases[1].

Oxidative stress is carried on by inflammatory processes, which lower cellular antioxidant capability. Free radicals that are overproduced interact with the fatty acids and proteins in cell membranes, irreversibly affecting their function[2]. Oxidative stress further worsens inflammation with the two related to each other in a positive feedback fashion. Further, Oxidative stress contributes to endothelial cell activation which is an early step in endothelial dysfunction[3]. Hence, chronic inflammation underlies endothelial dysfunction directly and via oxidative stress-related mechanisms. This implies that inflammation and the development of cardiovascular illnesses have been associated with an etiological network that includes interconnected elements such as oxidative stress and endothelial dysfunction[4].

Diet has a key role in predicting the levels of inflammatory markers found in the bloodstream. Saturated fatty acids (SFAs) and trans fatty acids, pro-inflammatory components, have continuously been linked to oxidative stress and proliferation, both of which may cause inflammation. Contrarily, it has been shown that monounsaturated fatty acids, PUFAs (polyunsaturated fatty acids), and fiber, reduce the inflammatory cascade. The adage “you are what you eat” underscores the importance of diet/food in the general

health and well-being of an individual. A healthy diet has been linked to a decreased incidence of cardiac illnesses. The underlying mechanisms are not yet clear[5]. There is evidence to support the idea that both acute and chronic inflammation may be controlled by diet, nutrients, and non-nutrient food components[1].

Foods are consumed in different combinations and proportions that form a whole diet or dietary patterns. Only a few research have looked at how different food patterns affect biological measurements of inflammation, oxidative stress, and endothelial function. The dietary patterns also vary by region. Hence it is essential to understand the impact of different dietary patterns particularly vegetarian and nonvegetarian have on inflammation in turn oxidative stress and endothelial functions.

The present study is undertaken to assess/compare the levels of inflammatory biomarkers, and oxidative stress markers and evaluate endothelial functions among vegetarians and non-vegetarians in this part of the country.

REVIEW OF LITERATURE

Dietary Pattern

The quantity, proportions, variety, or mix of various foods, beverages, and nutrients in diets, as well as the regularity with which they are consumed, are referred to as dietary patterns. Numerous societal, demographic, and personal factors might affect food habits[6].

TYPES OF DIETARY PATTERNS

Vegetarian diet: Vegetarianism spans a range of eating habits, from diets that exclude all animal foods and products (vegan) to diets that include eggs, milk, and milk products (lacto-ovo vegetarian), or even diets that include fish in addition to eggs, milk, and milk products, (pes-co-vegetarian). Veganism is a more stringent form of vegetarianism that completely forbids the consumption of animal products[7].

Non-vegetarian diet: A non-vegetarian diet includes all forms of plant foods as well as foods and by-products that are derived from animals, such as poultry, fish, meat, etc[8].

Mediterranean diet: accompanied by consumption of vegetables, legumes, fruits, and cereals; increased consumption of unsaturated lipids (predominately in the form of olive oil); low consumption of essential fatty acids; high average consumption of fish; low and moderate amounts of dairy products (mostly cheese or yogurt); low consumption of meat and poultry; and regular moderate levels of alcohol, primarily through the consumption of wine and typically during the meals[9].

Ketogenic diet: A ketogenic diet mostly consists of high fats, moderate proteins, and extremely few carbs. The ratios of fat, protein, and carbohydrates in the diet are

correspondingly between 55% and 60%, 30% and 35%, and 5% and 10%. For instance, a diet containing 2000 kcal per day may contain 20 to 50 g of carbohydrates[10].

Western diet: Red meat, cured/processed meat, and cured/processed red meat are frequently consumed in the Western diet pattern, which is characterized by a high intake of meat, highly processed foods, and sugars[11].

Dash diet: The Dietary Approaches to Stop Hypertension (DASH) diet was primarily created to lower blood pressure. A healthy diet with a low glycemic index and a low energy density. This dietary pattern discourages the consumption of red or processed meat, sugar, and sodium while containing a wide range of high-quality foods high in antioxidants, magnesium, potassium, and dietary fiber[12].

Diet and inflammation

Inflammation is thought to be a pathophysiologic process that underlies persistent low-grade inflammation, and research has shown correlations between this condition and an elevated risk of several illness. Nutrients are rarely consumed in quarantine, and the collaborating impacts of many different components may have a coordinated influence. analyzing food habits might provide a more clinical and holistic method of analyzing diet-disease links[13]. The whole dietary plan seems highly promising for reducing the inflammation associated with metabolic syndrome. The pathophysiology of age-related infirmity and the main chronic diseases of developed societies, such as Type 2 Diabetes Mellitus, Alzheimer's disease, Cardiovascular Disease, and different forms of cancer, has recently evolved to depend on inflammation[14]. Clear correlations between diet and inflammation have been identified after extensive research.

The inflammatory response is a sophisticated biological defense mechanism to combat physical, environmental, and pathological threats. The innate immune system may be stimulated by dietary patterns high in refined sugar and starches, trans, and saturated fatty acids, low in natural fiber and antioxidants from fruits, vegetables, and whole grains, and low in omega-3 fatty acids. This is most probably due to excess production of proinflammatory cytokines and a reduction in the production of anti-inflammatory cytokines. In comparison to non-vegetarian dietary patterns, vegetarian diets often include larger amounts of seeds, vegetables, nuts, fruits, legumes, and whole grains, all of which naturally contain higher levels of certain vitamins and phytochemicals. The vegetarian diet includes a variety of anti-inflammatory nutrients[15]. The beneficial ingredients may reduce circulating levels of inflammatory cytokines and improve inflammatory processes, consequently lowering the risk factor for age-related illness[13].

Many studies reveal that vegetarians experience less inflammation than non-vegetarians however, some studies did not detect a difference between the groups [16].

Inflammation and oxidative stress

Many biochemical systems, such as cell signaling, that are essential for physiologic cell function are based on redox reactions. Antioxidants and oxidants are important components of the processes. An oxidant is a substance that takes electrons, whereas an antioxidant is a substance that donates electrons. Under healthy circumstances, the production of oxidants and their elimination by antioxidant-scavenging chemicals are in equilibrium. In order for cells to operate normally, oxidants

are required for the oxidation, carboxylation, and hydroxylation processes, as well as the generation of prostaglandins, and deoxyribonucleotides. The imbalance between antioxidant defense and oxidant production, which increases oxidant concentration, is known as oxidative stress [17].

It has been demonstrated that oxidative stress-induced oxidation of plasma cysteine (Cys) and its disulfide cystine (CySS) increases the expression of the proinflammatory cytokine IL-1, induces monocyte adherence to vascular endothelial cells, and activates NF-B. Experimental studies actually demonstrate the co-existence of low-grade chronic inflammation and oxidative stress in a wide range of chronic diseases, including diabetes complications, cardiovascular and neurological disorders, alcoholic liver disease, and chronic kidney disease[18]. The pathogenesis and development of these diseases are strongly influenced by the activation and regulation of many signaling pathways by oxidative stress and inflammation [19]. Inflammation and oxidative stress are two processes that are directly connected to almost all diseases [20].

Chronic inflammation or inflammation that lasts too long can be dangerous and even cause disease[21]. At the site of the inflammation, inflammatory cells release various reactive species, intensifying oxidative stress. Many reactive oxygen/nitrogen species can start an intracellular signaling cascade that increases the expression of pro-inflammatory genes[18].

According to recent research, oxidative stress is connected to both diet and physical variables. Antioxidants are largely obtained from food. In regulating oxidative stress and its effects on the endothelium, dietary and environmental variables may play a

conflicting role. It has been shown that dietary factors can significantly alter the responsiveness of blood vessels. There has been a great deal of interest in using naturally occurring antioxidants and dietary treatments to treat or prevent metabolic and cardiovascular disorders because of the significant role that reactive oxygen species play in cardiovascular disease [22].

The macronutrients and micronutrients included in the diet are factors that can lead to the development of metabolic disorders such as type 2 diabetes, cardiovascular disease, metabolic syndrome, and other chronic non-communicable diseases (CNCDs)[23]. Inadequate intake of B-group vitamins has been linked to higher levels of cerebral inflammation and oxidative stress, as indicated by elevated blood plasma homocysteine, and is necessary for a healthy body and brain function[24]. Consuming meals which are high in antioxidants can assist in minimizing the negative consequences of oxidative stress. These healthful food elements and their byproducts help regulate the gut flora, which has an anti-inflammatory impact[25].

Inflammation, Oxidative Stress, and Endothelial function

Reactive oxygen species are produced more frequently during an inflammatory response, which eventually leads to endothelial dysfunction. The development of endothelial dysfunction is significantly influenced by inflammatory circumstances, and interleukin-1 (IL-1) is one of the main proinflammatory cytokines.

The endothelium is a highly sophisticated tissue that controls vascular homeostasis. The endothelium's ability to generate proinflammatory cytokines and adhesion molecules is fundamentally linked to the chronic vascular inflammatory process. Its primary role

includes controlling platelet aggregation, leukocyte adhesion, vascular tone, and smooth muscle cell development[26].

The endothelium takes an important part in modifying vascular tone by producing and distributing a variety of endothelium-dependent hyperpolarization (EDH) factors, in addition to endothelium-derived contracting factors. Decreased synthesis or activity of relaxing substances produced from the endothelium is the prime source of endothelial dysfunction, which can be a precursor to cardiovascular disease[27].

Endothelial dysfunction is caused by a variety of complicated and heterogeneous causes, including smoking, dyslipidemia, hyperhomocysteinemia, diabetes mellitus, arterial hypertension, cerebrovascular disorders, coronary artery disease, and heart failure[28].

Many research conducted on humans has linked higher inflammation to declined NO availability, demonstrating that infections or chronic inflammation might impair endothelial function. For instance, after administering young people with proinflammatory agents acutely, researchers looked into variations in endothelial function[26].

Oxidative stress and inflammation may be associated with a number of chronic disorders. For instance, the chronic vascular disease atherosclerosis is characterized by endothelial dysfunction (ED), oxidative stress, and inflammation. The pathogenesis of several diseases seems to share oxidant stress and an abnormal balance between oxidants and antioxidant defense systems. Systems like glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase, glutathione reductase, important trace elements,

and vitamins A, E, and C4 are examples of endogenous defense against oxidative damage. Lipid peroxidation and the generation of malondialdehyde (MDA) are caused by oxygen radicals because endogenous mechanisms are unable to offer the sufficient antioxidant capability. Inflammation can result from oxidative stress, Endothelial Dysfunction, and direct cellular injury. Inflammation, oxidative stress, and endothelial dysfunction are interconnected elements that may have a significant impact on the morbidity and mortality linked to a variety of chronic diseases. This in turn implies that strategies that control these variables might lessen the negative effects of chronic illnesses on both life quality and premature death[29].

Nitric oxide – As a marker of endothelial function

Between the vessel lumen and the vascular smooth muscle cells lies a monolayer of cells called the vascular endothelium. It is biologically active and generates different types of vasoactive cytokines, therefore it cannot be considered inactive. The regulation of vascular homeostasis depends on endothelial-derived nitric oxide, and abnormalities in the L-arginine: nitric oxide pathway has been linked to a number of cardiovascular illnesses.

The endothelial isoform of nitric oxide synthase converts the amino acid L-arginine into endothelium-derived nitric oxide, which produces L-citrulline as a by-product. In biological solutions, nitric oxide has a short half-life of only 4 seconds, making it labile. It is quickly converted by oxygenated hemoglobin to nitrite and subsequently nitrate prior to getting eliminated by the urine. Nitric oxide production requires a number of co-factors. They include calmodulin, flavin mononucleotide, flavin adenine dinucleotide,

tetrahydrobiopterin (BH4), and nicotinamide adenine dinucleotide phosphate (NADPH). Nitric oxide produced, crosses the endothelial cell membrane, and penetrates vascular smooth muscle cells in which it inhibits guanylate cyclase and raises intracellular concentrations of cyclic guanosine 3', 5-monophosphate (cGMP). Numerous biological effects of nitric oxide are augmented via cGMP as a second messenger, along with the regulation of platelet activity and vascular tone. Nitric oxide also targets DNA, thiols, haem or other iron-centered proteins, as well as other molecules. The functionality of some important enzymes or ion channels may change as a result of these new processes. Furthermore, nitric oxide binds with complex I and II, aconitase, and other enzymes of the respiratory chain to modulate tissue mitochondrial respiration. Because of the production of peroxynitrite ($ONOO^-$), a potent oxidant molecule, the combination of nitric oxide with superoxide anion can decrease physiological arousal caused by nitric oxide and have persistent inhibitory effects on mitochondrial activity.

Nitric oxide synthase (NOS) has three known isoforms: endothelial (eNOS), neuronal (nNOS), and macrophage or inducible (macrophage) (iNOS). In the control of vascular tone, each of the three NOS isoforms has a specific function. Chromosomes 7, 12, and 17 are home to the genes that code for eNOS, nNOS, and iNOS, respectively. While iNOS is not often expressed in vascular cells and is primarily detected in settings of infection or inflammation, eNOS and nNOS are normal components of healthy cells.

Nitric oxide, which is produced by the endothelium and is a powerful vasodilator in the vasculature, works in harmony with many endothelium-produced vasoconstrictors as well as the sympathetic nervous system to control blood vessel tone. Nitric oxide also inhibits platelet aggregation, leucocyte movement, and cellular attachment to the

endothelium and reduces the growth and movement of vascular smooth muscle cells. Nitric oxide can also affect the formation of superoxide anion and prevent the activation and expression of certain adhesion molecules. Studies in experimental animals have shown that the destruction of endothelium-derived nitric oxide might create a vascular profile greater susceptible to atherogenesis[30].

Hs-CRP (High sensitive C reactive protein)

Inflammation (including low grade inflammation) can be measured using serum inflammatory biomarkers, the levels of which provide an indication of the inflammatory status in the body. C-reactive protein (CRP) is one such biomarker[31].

C-reactive protein belongs to the pentraxins family. As CRP is an acute phase protein produced by hepatocytes in response to proinflammatory mediators, mainly interleukin-6, it is frequently employed as a general indicator of inflammation[32]. It has a molecular weight of 1,20,000 daltons and is made up of five subunits, each of which has 206 amino acids[33].

High-sensitivity test methods with short response times for analysis have been accessible in the last ten years. Immunonephelometry, immunoturbidimetry, high-sensitivity enzyme-linked immunosorbent assay (ELISA), and resonant acoustic profiling (RAP) are examples of high-sensitivity test methods that may detect CRP with a sensitivity range of 0.01 to 10 mg/l. In the absence of obvious systemic inflammatory or immunologic diseases, these high-sensitivity tests aid in quantifying mild degrees of acute inflammation. It is possible to quantify Hs-CRP correctly from newborn or adult plasma due to commercial assays that have been standardized across multiple platforms.

The most often studied biomarker in the search for the optimal biomarker for predicting the risk of cardiovascular disease (CVD) globally is the Hs-CRP.

According to studies, the Hs-CRP has opsonizing qualities that boost the recruitment of monocytes into atheromatous plaques and cause endothelial dysfunction by inhibiting basal and triggered nitric oxide release. Additionally, it has been discovered that the Hs-CRP itself alters the way that LDL is taken up by macrophages and increases the activation of the adhesion molecules vascular endothelial plasminogen activator inhibitor-1 (PAI-1) and other adhesion molecules[34].

In reaction to acute infection, inflammatory diseases, trauma, and aging, serum levels of CRP are raised. Many, but not all, studies have suggested that higher serum Hs-CRP levels are a predictor of all-cause death[35]. Cardiovascular disease risk is identified by an increase in high-sensitivity C-reactive protein (Hs-CRP) levels. The American Heart Association (AHA) advises that the best way to estimate cardiovascular risk is to monitor the levels of high-sensitivity C-reactive protein (Hs-CRP), a known chronic inflammation marker, in addition to the known risk factors[36]. Many variables, including sex, age, waist size, systolic blood pressure, and waist circumference, can influence serum Hs-CRP levels. It was postulated that after a high-fat meal, various populations would respond differently to inflammation[37]. Hs-CRP levels of less than 1, 1 to 3, and greater than 3 mg/L are associated with lower, moderate, and higher cardiovascular risks, respectively[38].

Serum Malondialdehyde (MDA):

MDA can be produced either through a non-enzymatic mechanism or by an enzymatic pathway that is similar to thromboxane A2 and prostaglandins. It serves a number of biological functions in addition to being a biomarker of oxidative stress. MDA, which serves a variety of biological purposes, can also be thought of as a biomarker of the peroxidation of cell membrane fatty acids when it is created by an enzymatic process. It has the ability to operate as an inducer of collagen-gene expression in hepatic cells as well as a signaling messenger in the release of insulin. Yet, MDA produced through a non-enzymatic method would interact with other biomolecules including proteins, amino groups, and DNA, which would ultimately have a genotoxic impact. The putatively most mutagenic molecule among ROS end-products has been identified as MDA. In the present study Serum MDA has been estimated as a marker of oxidative stress [39].

Joel C Craddock et.al, 2019 conducted a study to evaluate the link between vegetarian-based dietary patterns and inflammatory and immunological markers. They concluded that vegetarian diets are related with reduced levels of C-reactive protein, fibrinogen, and total leukocytes in the blood.[13].

Jeenan Kaiser, et.al 2021 conducted a systematic review of studies evaluating the association between vegan diets and cardiovascular outcomes. Among the Western populations studied, evidence weakly demonstrates associations between vegan diets and the risk of CVDs, with the direction of associations varying with the specific CVD outcome tested[40].

Mohd Ashraf Ganie et.al., 2019 This study intended to compare the effects of plant-based vs animal-origin diets on plasma markers of inflammation in women with PCOS and healthy women. They find that women with PCOS who eat an Indian vegetarian diet had greater levels of pro-inflammatory and lower levels of anti-inflammatory markers than their age and BMI-matched healthy non-vegetarian counterparts[41].

Binita Shah et.al aimed to study the effects of a vegan versus American heart association recommended diet on Hs-CRP, as well as other markers of inflammation, glucometabolic markers, and lipid profiles in patients with established coronary artery disease (CAD). For patients with coronary artery disease on guideline-directed medical

therapy, a vegan diet was found to lower high-sensitivity C-reactive protein, a risk marker of adverse outcomes[43].

Jadwiga Ambroszkiewicz et.al, 2018 The goal of this study was to look at the serum adipokine profile in prepubescent vegetarian and omnivorous youngsters. Sixty-two vegetarian children and 55 omnivore children aged 5 to 10 years were investigated. They found that the vegetarian diet modifies the adipokine profile and may have an anti-inflammatory impact. Greater anti- to pro-inflammatory adipokine ratios expressed as adiponectin/leptin and omentin/leptin ratios may indicate a superior metabolic panel of adipokines in vegetarian children[44].

Juliane Menzel et.al, 2020 aimed to evaluate the connections of veganism with a broad spectrum of inflammatory biomarkers, contrasted to omnivores. They concluded that there were no significant differences between vegans and omnivores in any of the inflammatory biomarkers studied (high-sensitivity C-reactive protein (Hs-CRP), interleukin-18 (IL-18), interleukin-1 receptor antagonist (IL-1 RA), intercellular adhesion molecule-1 (ICAM-1), adiponectin, omentin-1, and resistin)[45].

Riddhi Shah et.al, 2020 conducted a pilot research to investigate the relationships of the Mediterranean diet and its components with these direct markers of inflammation and oxidative stress from endothelial cells in women. The findings suggest that numerous features of the Mediterranean diet may help to reduce oxidative stress and inflammation while also improving endothelial function[46].

Yukihito Higashi, et.al, 2009 This review focuses on recent research on the interplay of endothelial function and oxidative stress in cardiovascular disease. In humans,

increased ROS generation affects endothelial function. Increased oxidative stress, which inactivates Nitric Oxide, is one method by which endothelial function is reduced. In individuals with cardiovascular disease, an imbalance of decreased Nitric Oxide generation and increased Reactive Oxygen Species production may be implicated in poor endothelium-dependent vasodilation[47].

DIEGO MEZZANO et.al, **2001,** The purpose of this study was to measure plasma homocysteine (tHcy) and inflammatory response markers and analyze their relationships with coexisting endothelial dysfunction, oxidative stress, and hemostatic activation in patients with varying degrees of chronic renal failure (CRF) receiving conservative treatment. They found that systemic inflammation, which is linked to increased oxidative stress, endothelial cell dysfunction, and hemostatic activation, is a substantial cardiovascular risk factor[48].

Ute Alexy et.al 2021, conducted a study on the Vechi Youth Researchers analyzed anthropometric data, food consumption, and nutritional status among 149 vegetarian, 115 vegans, and 137 omnivore children and adolescents (6-18 years old, mean age: 12.7 3.9 years). They concluded that the Vechi Youth Study did not indicate specific nutritional risks among vegetarian and vegan children and adolescents compared to omnivores[49.]

The above reviews don't give a clear idea of the association between diet and inflammation, oxidative stress, and endothelial function. There is a paucity of studies conducted in this region of the country. In India, the diet is unique to the region and the various cultural factors influence the dietary pattern. Hence the present study is undertaken.

AIM

The present study is undertaken to assess the effect of different dietary patterns (vegetarian and Non-vegetarian) on the inflammatory marker, oxidative stress marker, and endothelial functions.

OBJECTIVES OF THE STUDY

1. To assess and compare inflammatory markers, oxidative stress markers, and endothelial functions among vegetarians and non-vegetarians.
2. To find an association between inflammatory markers, oxidative stress markers, and endothelial functions.

MATERIALS AND METHOD

SOURCE OF DATA: Study participants will be recruited from Shri B. M. Patil Medical College, Hospital and Research Center, BLDE (Deemed to be University), Vijayapura from among the teaching and non-teaching faculty volunteering to participate in the study.

TYPE OF STUDY: Cross-sectional comparative study.

STUDY DURATION: 6 Months

STUDY SUBJECTS: Apparently healthy subjects, aged 20-40 years, both men and women on either a vegetarian or non-vegetarian diet for at least 1 year. The study participant will be interviewed depending on their dietary pattern and classified into vegetarian and non-vegetarian depending on the following operation definition.

1. Vegetarians: defined as the absence of the consumption of meat, meat products, fish, and seafood [41].

2. Non-vegetarians: Subjects who consumed meat/chicken/fish at least twice in a week for the last year [42].

The study participants (n=90) will be divided into two groups as follows:

Group 1: Vegetarians (n=45)

Group 2: Non-Vegetarians (n=45)

INCLUSION CRITERIA: aged 20-40 years, following the diet (either vegetarian or non-vegetarian) for at least 1 year**.**

EXCLUSION CRITERIA: Cardiovascular disease, diabetes mellitus, cancer, pregnancy, current infection, chronic inflammatory diseases, H/O smoking, alcohol consumption, tobacco chewing, consuming medications like glucocorticoids, NSAIDs, etc known to affect inflammatory markers, any prior history (at least 2 weeks) of infection, trauma, a surgery known to generate an inflammatory response, refusal to participate in the study.

Ethical considerations:

Written informed consent will be obtained from the study participants before their enrolment in the study. Prior approval for the study will be obtained from the institutional ethical committee as per guidelines of the Indian Council of Medical Research (2017)[50]. The Declaration of Helsinki will be followed during the entire study.

METHODS OF DATA COLLECTION:

The participants recruited for the study will be interviewed for details of their dietary patterns. They will be classified as either vegetarians or non-vegetarians as per the operational definition. The study participants will be subjected to physical anthropometry, a recording of Physiological parameters. Blood sampling: Random Collection, samples will be centrifuged and serum separated and aliquoted and stored at (-20^0 C) for conservation until the time of analysis for inflammatory markers, oxidative stress markers and , markers of endothelial function.

The following parameters will be recorded

a. Age (years):

b. Sex: M F

c. Place of residence:

1. Physical anthropometry:

Physical anthropometry will be recorded while participants with light clothing and no shoes.

a. Height (meters):

b. Weight (kilograms):

c. Body Mass Index (kg/m^2):

2. Physiological Parameters

All the recordings will be done following a supine rest of 10 minutes.

a. Pulse rate (beats/min)

b. Systolic and Diastolic Blood Pressure (mm Hg) in the supine position

1. Diet History:

a. Vegetarians: defined as the absence of the consumption of meat, meat products, fish, and seafood[41].

b. Non-vegetarians: subjects who consumed meat/chicken/fish/egg at least twice a week [42].

2. Inflammatory biomarkers:

a. Serum Hs-CRP: Will be assayed by ELISA using commercially available kits and according to the manufacturer's protocol.

3. Oxidative stress markers

a. Serum Malondialdehyde (MDA): Serum MDA will be estimated by the method of Buege and Aust [51].

4. Measures of endothelial function:

a. Serum Nitric oxide: Serum Nitric oxide will be estimated by Greiss reaction [52,53].

1. Estimation of Malondialdehyde[51]:

By the method of Buege and Aust (Buege and Aust, 1978)

● Introduction

Malondialdehyde (MDA) is employed as an indication of oxidative stress in biological systems. It is produced as a byproduct of lipid peroxidation. A free radical chain reaction degrades polyunsaturated fatty acid (PUFA) to create it. This substance is a reactive aldehyde and one of the numerous reactive electrophile species that cause oxidative stress.

• Principle

MDA, which is generated by the breakdown of PUFA, is a useful indicator for determining the amount of lipid peroxidation. It combines with thiobarbituric acid (TBA) to produce a pink color that may be measured at 535nm.

• Samples include serum, saliva, and tissue homogenate.

• **Chemicals needed:**

1. (TCA) Trichloroacetic acid
2. (TBA) 2-Thiobarbituric acid
3. (HCl) Hydrochloric acid
4. (dimethyl acetal) Malonaldehydebis

● Preparation:

1. Reagent TCA-TBA-HCl

0.25 N HCl: 2.21 mL of concentrated HCl is mixed with distilled water to make 100 mL. (DW). In 100ml of 0.25N HCl, dissolve 15% TCA and 0.375% TBA. The reaction mixture is warmed to dissolve the ingredients and then stored at 40 degrees Celsius.

1. MDA standard (stock-164µg/ml)

16.4µl of standard MDA solution is used and distilled water is added to make 100 ml.

2. MDA functioning standard (working- 1.64µg/ml)

Using distilled water, 100µl of stock is created up to 10 ml.

- **The standardizing procedure:**

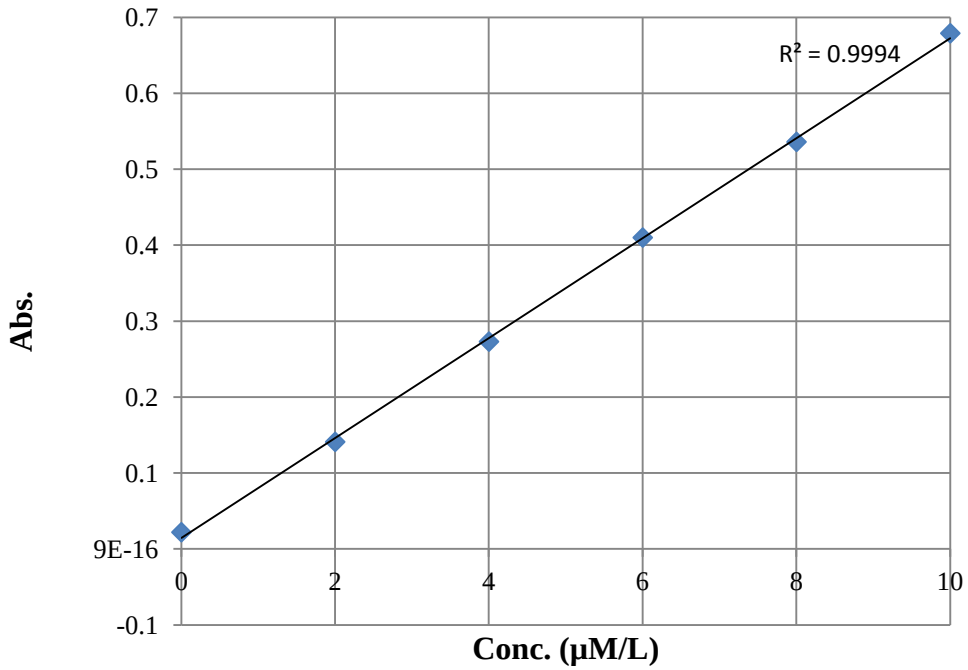
Standardization (from 2 to 10 M/L)

The standardization is conducted using the table, and all of the reaction mixtures are added in accordance with the measurement indicated in the table.

S.No.	Vol. of MDA (ml)	Vol. of DW (ml)	Conc. of MDA (µM/L)	TBA-TCA-HCl (ml)	Keep in boiling water bath for 15 min
B	0.0	1	0.0	1	
1	0.2	0.8	2.0	1	
2	0.4	0.6	4.0	1	
3	0.6	0.4	6.0	1	
4	0.8	0.2	8.0	1	
5	1	-	10.0	1	

Read O.D. absorbance at 535 nm. The optical densities are plotted against the concentration on a graph.

● Standard Curve



MDA estimation in the sample:

• Sample preparation:

Serum: Distilled water is used to dilute 100µl of serum to 500µl.

Tissue Homogenate: 500µl of 10% tissue homogenate

(Tissue homogenate preparation: 10% tissue homogenate was made by mixing 500 mg of tissue with 5 ml of 0.1M phosphate buffer, homogenizing for a few minutes, centrifuging, and using the supernatant in the estimate).

● Procedure

1. Add 1 ml of TCA-TBA-HCl reagent to the diluted sample.

2. For 15 minutes, the samples are immersed in a boiling water bath.

3. Cool and centrifuge the reaction mixture.

1. The supernatant is collected, and the optical densities of the pink color generated are measured using a UV visible spectrophotometer at 535nm (Shimadzu, Model: UV 1800).

2. The concentration of MDA in the sample is determined by plotting the absorbance obtained against the standard graph. The optical density of the generated pink hue is related to the concentration of MDA in the sample.

● Calculation

The optical densities of the test samples are directly proportional to the concentration of MDA in the sample and calculated by plotting against the standard graph and multiplying by the respective dilution factors. The final concentration is expressed as µM/L.

Serum Nitric Oxide Level evaluation[52,53].

By Greiss Reaction (Moshage Han et al., 1995; Cortas and Wakid, 1990; Green et al., 1982)

• Principle

The stable byproduct of nitric oxide, nitrate, was coupled to N-naphthyl ethylene diamine and then reduced to nitrite using the cadmium reduction process. A spectrophotometer was used to measure the colored complex that was created at 540 nm.

• Reagents

1. Granules of cadmium: 2.5–3 gm in 0.1 M/L H2SO4

2. Glycine-NaOH buffer (pH-9.7): 200 milliliters of distilled water were used to dissolve 7.5 grams of glycine. The pH was then brought down to 9.7 by adding 2M NaOH, and 500 ml of distilled water was used to dilute it.

3. Sulfanilamide: 2.5 grams of sulfanilamide were dissolved in 250 milliliters of heated 3M/L HCl and then allowed to cool.

1. N-Naphthylethylene diamine: 50 mg of N-Naphthylethylene diamine was dissolved in 250 ml of distilled water.

2. A stock standard of sodium nitrite solution (0.1 mol/L)

In 100 ml of a sodium borate solution containing 10 mmol/L, 690 mg of sodium nitrite was dissolved.

b. Workplace standard (10 mol/L)

10 mmol/L of sodium borate solution was used to dilute a stock solution of sodium nitrite (NaNO2) in 10 l up to 100 ml.

3. A 75 mmol/L solution of ZnSO4

4. A 55 mmol/L solution of NaOH

5. A 0.1 mol/L solution of H2SO4

6. A 5 mmol/L solution of CuSO4

The glycine-NaOH buffer was used to dissolve 125 mg of CuSO4 in 100 ml.

- Step a. Deproteinization: 0.5 ml of serum was obtained and placed in a clean, dry centrifuge tube. This was combined with 2.0 ml of a 75 mmol/L ZnSO4 solution. After adding 2.5 ml of the 55 mmol/L NaOH reagent and thoroughly mixing it in, the sample was centrifuged for 10 minutes. The supernatant was handled as a filter devoid of protein.

- Step b. Activation of cadmium granules: At the time of the test, cadmium granules that had been previously kept in 0.1mol/L H2SO4 solution were three times rinsed with distilled water. The granules were then stirred for 1-2 minutes in a 5 mmol/L CuSO4 solution. The glycine-NaOH buffer was used to drain and wash the copper-coated granules. After activation, these granules were consumed within 10 minutes. After usage, the granules were rinsed with distilled water and kept in a 0.1 mmol/L H2SO4 solution. Each time, the exact identical granule activation process was used.

- **Nitric test**:

1. Three Erlenmeyer flasks were obtained and labeled as Blank (B), Test (T), and Standard for the nitrite assay (S).

Each Erlenmeyer flask received 1 ml of glycine-NaOH buffer. 1 ml of deionized water, 1 ml of the deproteinized sample, and 1 ml of the working standard were added to the flasks marked B (Blank), T (Test), and S (Standard), respectively.

2. Freshly activated cadmium granules weighing 2.5 to 3 grams each were added to each flask using a spatula.
3. The granules were mixed in all of the flasks' contents.
4. The mixture in each of the three flasks was diluted to 4ml with distilled water after 90 minutes.
5. From the appropriate flasks, 2 ml of this solution was pipette into three clean, dry test tubes with the letters B, S, and T, accordingly.
6. Each tube received 1 ml of sulfanilamide and 1 ml of N-naphthyl ethylene diamine solution.
7. The OD of S and T were read against a blank at 540 nm using a spectrophotometer after 20 minutes of vigorous shaking of all three tubes.

● Calculations

$$\text{Serum Nitric Oxide (µmol/L)} = \frac{OD\ of\ Sample}{OD\ of\ Standard} \times conc.oftandard \times DF$$

Estimation of Hs-CRP[54].

1. Immediately separate the serum after collecting blood samples.

2. Normally, samples can be kept chilled at (2–8°C) for 5 days. For storing items longer than five days, keep them frozen at (-20°C) for up to one month.

3. Avoid performing frequent freeze-thaw cycles.

4. Frozen sera must be thoroughly thawed and mixed before being used in an experiment.

5. Avoid using severely lipemic specimens.

PREPARE THE REAGENTS Wash Buffer, 1X: By mixing 475 ml of distilled or deionized water with the contents of the bottle (25 ml, 20X), 1X Wash buffer can be made. Storage at 20 to 25 C room temperature. TESTING PROCEDURE Let the reagents to stand at room temperature (20–25 °C) before the test. Before using, thoroughly combine all reagents.

1. Fill the holder with the required number of coated strips.

2. Add 5 l of sample to 495µl of sample diluent to dilute patient samples and controls at a ratio of 1:100. (STANDARDS ARE READY TO USE).

3. Fill the relevant wells with 10µL of standard, diluted samples and controls.

4. Fill each well with 100µl of enzyme conjugate. To eliminate air bubbles from the liquid and thoroughly combine, tap the holder.

5. Incubate for 60 minutes at 20 to 25 C room temperature.

6. Empty all wells of liquid. Wash the wells three times in 1X wash buffer using 300µl. On absorbent paper towels, blot.

7. Fill each well with 100µl of TMB substrate.

8. Incubate at room temperature for 15 minutes.

9. Fill each well with 50µl of stop solution. To combine the solution, gently shake the plate.

10. After adding the stopping solution, check the absorbance on an ELISA Reader at 450 nm within 15 minutes.

ARRANGEMENT OF RESULTS

The following is how the standard curve is created:

1. Verify each standard vial's CRP standard value. This number might change from lot to lot. Verify each kit's value by checking it. See the linked standard example.

2. On a piece of linear graph paper, plot the absorbance of the CRP standards (vertical axis) vs the concentrations of the CRP standards (horizontal axis) to create the standard curve. Between the points, trace the best curve.

3. Take the curve's absorbance values for each unknown sample and the controls. For every control sample or unidentified sample, note the value.

4. To get CRP readings in mg/l, multiply the values from the patient samples and control sera by the 100-fold dilution factor.

5. After the initial 100-fold dilution, patient samples with CRP concentrations over 10 mg/l should be further diluted by 10 times (total dilution: 1: 1,000), and the final CRP values should be multiplied by 1,000 to produce CRP results in mg/l.

SAMPLE SIZE

- With anticipated Mean±SD of Serum hs-CRP in the vegetarian group 2.19±1.48 and in the non-vegetarian group 1.68±1.52 resp. [8] the required minimum sample size is 45 per group (i.e. a total sample size of 90, assuming equal group sizes) to achieve a power of 80% and a level of significance of 5% (two-sided), for detecting a true difference in means between two groups, using G* Power 3.1.9.7.

STATISTICAL ANALYSIS

- The data obtained will be entered into a Microsoft Excel sheet, and statistical analysis will be performed using a statistical package for the social sciences (Version 20).
- Results will be presented as Mean±SD, counts and percentages, and diagrams.
- For normally distributed continuous variables between two groups will be compared using an Independent t-test For not normally distributed variables Mann Whitney U test will be used. The correlation between inflammatory markers will be analyzed using Pearson's /Superman's correlation.
- p<0.05 will be considered statistically significant. All statistical tests will be performed in Two-tailed.

RESULT

Table-1.

Comparison of anthropometric parameters among vegetarians and non-vegetarians.

Variables	Group 1 Vegetarian (n=45)	Group 2 Non-Vegetarian (n=45)	P value
Age (Years)	28.42 ± 10.38	26.27 ± 7.817	0.269
Height (Cm)	167.24 ± 7.71	169.16 ± 7.211	0.228
Weight (Kg)	55.24±6.81	58.16 ± 6.856	0.046*
BMI	19.702 ±1.72	20.311 ± 1.8754	0.139

Table 1: depicts a comparison of anthropometric parameters among vegetarian and non-vegetarian subjects. Subjects of both groups were matched for age, hence there was no significant difference in age. There was no significant difference in the height of the subjects The weight of the subjects in group 2 (non-vegetarians) was significantly (p-0.046) higher compared to group 1 (vegetarians). No significant difference in the BMI of the subjects of group 1 and 2.

Table-2.

Comparison of physiological parameters among vegetarians and non-vegetarians.

Variables	Group-1 Vegetarian (n=45)	Group-2 Non-Vegetarian (n=45)	P value
Pulse rate (bpm)	87.93 ± 7.168	87.60 ± 7.779	0.833
SBP (mmHg)	111.87 ± 7.494	111.98 ± 7.362	0.944
DBP (mmHg)	78.09 ± 5.612	78.47 ± 5.582	0.750

Table 2: depicts the comparison of physiological parameters among vegetarian and non-vegetarian individuals. There was no significant difference in the pulse rate of the subjects. Both systolic blood pressure and diastolic blood pressure, represent no significant differences among vegetarian and non-vegetarian groups.

Table-3.

Comparison of biochemical parameters among vegetarians and non-vegetarians.

Variables	Group-1 Vegetarian (n=45)	Group-2 Non-Vegetarian (n=45)	P value
Hs-CRP (mg/L)	0.029 ± 0.050	0.026 ± 0.016	0.319
Serum NO (mmol/L)	22.11 ± 17.06	24.67 ± 22.850	0.641
Serum MDA (µM/L)	1.88 ± 1.21	0.576 ± 1.0306	0.000*

*p<0.05

Table 3: This table compares biochemical parameters among vegetarians and non-vegetarians. Hs-CRP of the subjects depicts no significant variations. There was no significant difference in Serum NO. Serum MDA was significantly higher among group 1 (vegetarians) compared to group 2 (non-vegetarians).

Table-4.

Correlation of Age and Biochemical parameters among vegetarians and Non-vegetarians

Vs. Age	Vegetarian (n=45)		Non vegetarian (n=45)	
	R-value	P-value	R-value	P-value
Hs-CRP(mg/L)	0.285	0.058	0.0261	0.083
Serum MDA (µM/L)	-0.349*	0.019	0.459	0.002*
Serum NO (mmol/L)	-0.065	0.672	-0.115	0.452

*p<0.05

Table-4. Depicts the Correlation of Age and Biochemical parameters among group 1 (vegetarian) and group 2 (Non-vegetarian). There was no significant correlation between age and Hs-CRP, among group 1 (vegetarians) and group 2 (Non-vegetarians). In MDA with age, at the 0.01 level (2-tailed), P=0.019 and R=0.002 in vegetarians and non-vegetarians, respectively, have shown a significant correlation. The correlation of age and NO among vegetarians and non-vegetarians was shown no significant correlation.

Fig:1

Correlation between age and Serum MDA in Vegetarians

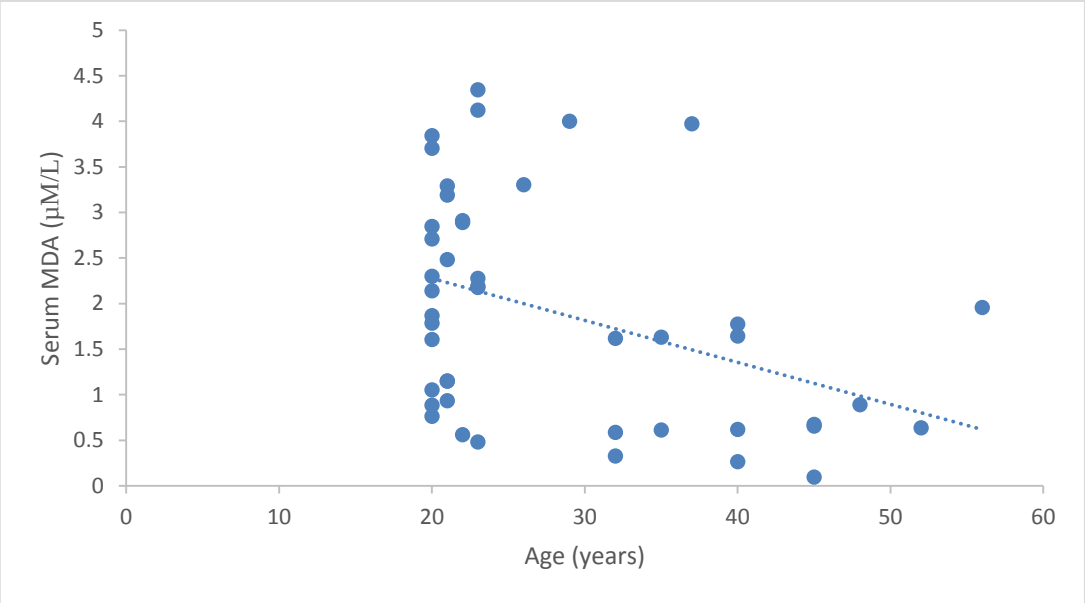


Fig:2

Correlation of Age with MDA in Non-vegetarians

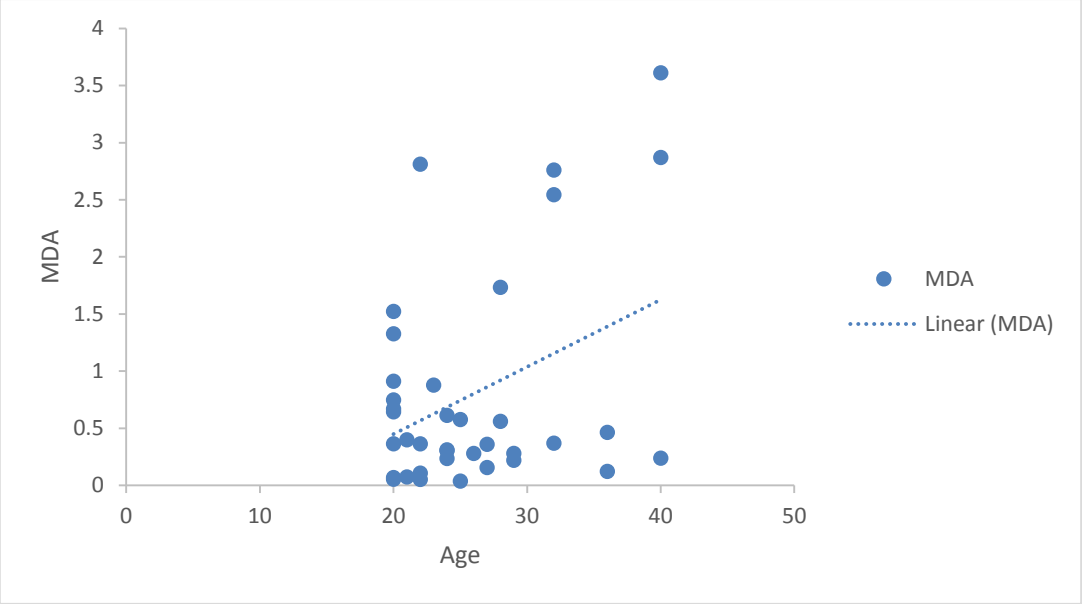


Table-5.

Correlation between BMI and Biochemical parameters among vegetarians and Non-vegetarians

Vs. BMI	Vegetarian (n=45)		Non vegetarian (n=45)	
	R-value	P-value	R-value	P-value
Hs-CRP (mg/L)	0.185	0.223	0.079	0.605
MDA (µM/L)	0.113	0.460	0.045	0.767
NO (mmol/L)	-0.048	0.755	-0.178	0.243

Table-5. Determines the correlation between BMI and Hs-CRP, Serum MDA, and Serum NO among vegetarians and non-vegetarians. On analysis, no significant correlation was detected.

Table-6:

Correlation between Hs-CRP with MDA and NO

VS Hs-CRP	Group-1 Vegetarians (n=45)	Group-2 Non-vegetarians (n=45)

	r-value	p-value	r-value	p-value
MDA	-0.059	0.700	0.133	0.383
NO	-0.157	0.304	-0.001	0.993

Table 6: Depicts that there is no significant correlation between Hs-CRP with MDA and Nitric oxide.

Discussion

The present study aimed to assess inflammatory biomarkers, oxidative stress markers, and endothelial functions among vegetarians and non-vegetarians. The two groups were matched for age and gender. On anthropometric analysis the body weight was higher in non-vegetarians compared to vegetarians. However, the BMI was comparable between vegetarians and non-vegetarians. In contrast to the results of the present study, Barnard et al., in their study demonstrated a significant lower weight and BMI in subjects on a vegetarian diet[63]. In the present study the physiological parameters like pulse rate, systolic and diastolic blood pressure did not vary between vegetarians and non-vegetarians. In contrast to findings of the present study, Fraser G et al. demonstrated considerably lower systolic and diastolic blood pressure in vegetarians when compared to non-vegetarians. The results of their study also demonstrated that vegetarians had a significantly reduced probability of developing hypertension compared to non-vegetarians[64].

Hs-CRP was assessed as an inflammatory biomarker. The results of the present study demonstrated that Hs-CRP was comparable between vegetarians and non-vegetarians. Further, there was no correlation between Hs-CRP, BMI, and age. Similar results were obtained by Haghighatdoost et al., in their study. While some studies showed that Hs-CRP increases with increased body mass[65]. There are studies demonstrating that vegetarians have lower levels of Hs-CRP than non-vegetarians[13]. others demonstrated the beneficial effect of fruit and vegetable intake on inflammatory marker[66].

The anti-inflammatory effects of vegetarianism may be mediated by several substances in vegetarian diets, including phytosterols, spices, salicylic acid, and dietary fiber. Phytosterols reduce the production of inflammatory mediators. A few common spices in vegetarian diets have an anti-inflammatory impact by inhibiting the formation of pro-inflammatory markers. Dietary fiber and salicylic acid are active components of anti-inflammatory drugs and are known to be present in fruits and vegetables[67].

In the present study, informal enquiry by the investigator about the types of food intake revealed that non-vegetarians consumed a good amount of vegetables and fruits apart from the meat consumption. Also, it is reported that the levels of inflammatory biomarkers are influenced by the type of meat consumed. Fish consumption lowers biomarkers of inflammation, while red meat raises inflammation[67]. In the present study, the type of meat consumed has not been taken into account and has not been evaluated. Also there are several other factors that influence the level of inflammatory biomarkers like level of physical activity, intake of junk food, etc which have not been analyzed in

the present study. This could be the reason, why there were no significant differences in the Hs-CRP levels among vegetarians and non-vegetarians.

Malondialdehyde (MDA) is one of the by-products of peroxidation of polyunsaturated fatty acid in the cells[68]. A rise in free radicals leads to an excess of MDA generation. The presence of malondialdehyde is frequently used as a sign of oxidative stress and antioxidant status[69]. In the present study, interestingly serum MDA levels were significantly higher in vegetarians compared to non-vegetarians. Again informal enquiry by the investigator revealed that the food variety/good groups and consumption of fruits, vegetables including greens was limited among vegetarians. However, non-vegetarians consumed variety of fruits, vegetables in addition to the non-vegetarian diet. This warrants further study and detailed analysis of the food groups among vegetarians and non-vegetarians. On correlation, Serum MDA was positively correlated with age ($r=0.459$,$p=0.002$) in non-vegetarians while negatively correlated with age ($r=-0.349$, $p=0.019$) among vegetarians. The food choices, food groups and intake in terms of quality and quantity may be affected by the age of the subject. Also oxidative stress is higher in sedentary, stressful life and with bad eating habits such as high calorigenic food and type of meat consumption, junk food, fast foods, processed foods[70]. One of the studies indicated that the difference in MDA levels between vegetarians and non-vegetarians was negligible, and the high intake of fast foods, processed foods, meat, etc., aggravates oxidative stress irrespective of being a vegetarian or a non-vegetarian. Since the participants in the present study include young adults to middle aged, this could have an influence on food choices, food groups and intake in terms of quality and quantity. Hence, further study on the influence of age on food choices, food groups and food

intake in terms of quality and quantity, level of physical activity among vegetarians and non-vegetarians is required. Further there was no significant correlation between Serum MDA and BMI among both vegetarians and non-vegetarians. Studies revealed that MDA levels increased with age[70]. Aging is a steady loss in biochemical and physiological function that occurs over time and is linked to a higher risk of death and morbidity. Oxidative stress (OS) is being more recognized as significantly impacting the aging process and several degenerative illnesses like Alzheimer's, cancer, diabetes, and chronic inflammation. According to the OS theory, originally put out by Harman in 1956, age-related biochemical and physiological deterioration is linked to a persistent state of imbalance between the generation of oxidants and the capacity of intracellular antioxidants. This may result in various cellular processes changing negatively, impairing metabolic performance[71].

Serum NO is used as a marker of endothelial function. The regulation of vascular homeostasis depends on endothelial-derived nitric oxide levels, and reduction in the NO levels causes arterial stiffness that leads to hypertension and other cardiovascular complication. As we compared levels of nitric oxide in vegetarians and non-vegetarians, there were no significant differences among vegetarians and non-vegetarians. These results are in accordance with a study by Zuelch and Michelle[72]. Ramos et al., reported that the arterial stiffness and endothelial functions are comparable between vegetarians and omnivores[73].

In the present study no correlation was observed between, HsCRP, Serum MDA and Serum NO among vegetarians and non-vegetarians. Studies have depicted the role of inflammation and oxidative stress in endothelial dysfunction. Inflammatory processes

induce oxidative stress and reduce cellular antioxidant capacity. Overproduced free radicals react with cell membrane fatty acids and proteins impairing their function permanently[2]. Oxidative stress further worsens inflammation with the two related to each other in a positive feedback fashion. Further, Oxidative stress contributes to endothelial cell activation which is an early step in endothelial dysfunction[3]. Hence, chronic inflammation underlies endothelial dysfunction directly and via oxidative stress-related mechanisms. This implies that inflammation, oxidative stress, and endothelial dysfunction are interrelated components of an etiological network that has been linked to the development of cardiovascular disease. However, further studies with greater sample size are required to validate the findings of the present study of no correlation between HsCRP, Serum MDA and Serum NO.

Conclusion: The results of the present study indicate an impact of vegetarian and non-vegetarian dietary patterns on oxidative stress with a better oxidative stress profile among non-vegetarians. The results of the present study warrant a detailed analysis of the adequacy of food groups, food variety, quality, and quantity of different food components. Also, there is a need to understand how food preferences change with age. Further studies are also needed to delineate the influence of lifestyle factors like a sedentary lifestyle, and levels of physical activity on markers of inflammation, oxidative stress, and endothelial functions in addition to dietary patterns.

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BLDE

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The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 714/2022-23 30/8/2022

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Friday, 26th August, 2022 at 3.30 p.m. in the Department of Pharmacology** scrutinizes the Synopsis of Post Graduate Student of BLDE (DU)'s Shri B.M.Patil Medical College Hospital & Research Centre from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "Association of Dietary Patterns, with inflammatory Biomarkers, Oxidative Stress and Endothelial Function".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: Miss Muskan page, MSc Clinical Immunology.

NAME OF THE GUIDE: Dr. Shrilaxmi Bagali, Associate Professor, Dept of Physiology.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA

Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA

MEMBER SECRETARY
Institutional Ethics Committee
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Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

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Informed Consent Form

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned,_____ , S/O D/O W/O _____, aged ___years, ordinarily resident of _____ do hereby state/declare that Dr _____ of _____ Hospital has examined me thoroughly on _____ at _____ (place) and it has been explained to me in my own language that I am suffering from _____ disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled

_____

_____

_____ under the guidance of Dr _____requesting my participation in the study.

The doctor has also informed me that during the conduct of this procedure, adverse results may be encountered. Among the above complications, most of them are treatable but are not anticipated hence there is a chance of aggravation of my condition and in rare circumstances, it may prove fatal in spite of the anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study will help in the evaluation of the results of the study which is a useful reference for the treatment of other similar cases in the near future, and also I may be benefited from getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by a person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on the information given by me, I can ask for any clarification during the course of treatment/study related to diagnosis, the procedure of treatment, the result of treatment, or prognosis. At the same time, I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of the dissertation or research, the diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my fully conscious state of mind agree to participate in the said research/dissertation.

Signature of Patient:

Signature of Doctor:

Witness: 1.
2.

Date:

SCHEME OF CASE TAKING

Name:

Age (years): Sex: M F

Occupation

Place of residence:

1. Physical anthropometry:

a. Height (metres):

b. Weight (kilograms):

c. Body Mass Index (kg/m^2):

2. **Physiological Parameters**

a. Pulse rate (beats/min)

b. Systolic and Diastolic Blood Pressure (mm Hg) in the supine position

3. Diet History:

a. Vegetarian

b. Non-vegetarian

4. Inflammatory biomarkers:

a. Serum hs-CRP:

5. Oxidative stress markers:

a. Serum Malondialdehyde (MDA):

6. **Measures of endothelial function:**

a. Serum Nitric oxide:

Vegetarian master chat

	sex	age	heightcm	weight	BMI	pulse rat	SBP	DBP	hsCRP	NO	MDA
	F	20	149	40	18	98	102	79	0.00344	13.3	0.76356
	F	20	161	46	17.7	87	107	75	0.01139	14.166	2.30021
	F	23	155	43	17.9	92	126	72	0.00132	9.166	0.48199
	F	21	162	48	18.3	89	110	80	0.00505	53.74	2.48324
	M	22	168	55	19.5	95	105	82	0.00781	16.25	0.56173
	M	21	171	57	19.5	94	115	72	0.02861	12.5	1.15029
	M	20	173	53	17.7	78	120	76	0.02702	11.25	1.05335
	F	20	168	50	17.7	85	130	73	0.02227	11.16	0.88585
	M	21	165	53	19.5	96	125	80	0.01366	17.91	1.15063
	M	21	169	55	19.3	93	122	79	0.00766	21.25	0.93449
	M	21	176	60	19.4	87	116	72	0.03216	15	3.19117
	M	22	173	59	19.7	76	109	75	0.00795	13.3	2.91097
	M	21	178	65	20.5	92	123	74	0.0013	23.75	3.29188
	M	20	171	60	20.5	89	102	80	0.03708	11.25	3.84303
	M	20	175	58	18.9	97	116	73	0.00165	17.91	3.70465
	F	26	161	55	21.2	78	114	72	0.04148	10.41	3.30489
	F	23	154	52	21.9	82	101	76	0.01429	29.166	4.12323
	F	37	170	68	23.5	96	105	75	0.03406	22.03	3.97354
	F	23	167	59	21.2	99	118	72	0.0013	70.83	2.27761
	M	20	167	60	21.5	91	104	78	0.01545	18.33	2.7099
	F	23	158	40	16	89	108	80	0.01152	35.24	2.19197
	F	20	161	44	17	75	111	90	0.02859	31.66	1.78571
	F	20	159	49	19.4	88	120	86	0.00153	32.5	2.84706
	F	20	164	52	19.3	90	110	80	0.3356	47.5	2.14093
	F	23	161	49	18.9	79	115	80	0.00631	31.25	4.34657
	M	23	170	55	19	95	107	90	0.00056	16.25	2.17758
	F	40	161	55	21.2	78	102	75	0.04696	15.41	0.61969
	F	37	168	53	18.8	82	100	85	0.05844	12.12	0.67484
	F	40	162	57	21.7	89	119	70	0.04771	83.33	0.65634
	F	39	179	62	19.3	76	116	80	0.01627	14.625	0.63716
	F	40	175	65	21.2	85	118	80	0.05906	12.625	1.95767
	M	32	161	55	21.2	78	106	90	0.04875	12.25	0.32784
	F	40	192	70	19	95	109	85	0.04699	13.125	0.89098
	M	40	172	57	19.3	94	103	78	0.01394	67.5	0.2655
	M	35	170	60	20.8	89	107	80	0.01694	12.08	0.61318
	M	32	173	57	19	75	118	74	0.00987	16.125	0.58715
	F	29	169	62	21.7	88	112	79	0.04851	12.9	4.00129
	M	22	172	52	17.6	79	110	72	0.01545	14.798	2.89042
	F	20	164	48	17.8	95	100	80	0.05371	21.25	1.86826
	F	40	164	52	19.3	90	108	90	0.00893	11.375	1.77543
	M	40	177	62	19.8	84	117	70	0.00471	12.87	0.09657
	F	40	172	56	18.9	85	106	85	0.01782	14.9	1.64461
	F	35	167	55	19.7	91	112	75	0.06025	20.83	1.63181
	F	32	158	58	23.2	99	116	73	0.03549	11.5	1.61901
	F	20	164.23	65	24.1	95	114	72	0.00338	11.08	1.60621

Non-Vegetarian master chat

sex	AGE	height(cr	weight k	BMI	pulse rat	s	d	hsCRP	NO	MDA
M	40	167.64	65	23.1	92	120	76	0.04882	13.33	2.87021
M	32	170.6	73	25.3	87	130	73	0.04591	15.41	2.76025
M	29	176.78	76	24.5	97	125	80	0.00338	12.12	0.21994
M	22	164.64	55	20.3	92	122	79	0.04148	83.33	2.81163
M	20	176.32	65	20.9	96	116	72	0.01429	14.625	0.05415
F	25	155.4	50	20.7	98	109	75	0.03406	12.625	0.57653
F	20	152.4	45	19.4	85	123	74	0.00647	12.25	0.91188
M	21	170.6	62	21.3	78	102	80	0.01543	13.125	-0.0092
M	20	179.83	65	20.1	82	116	85	0.02228	67.5	-0.1227
M	20	173.7	60	19.9	89	107	75	0.03052	12.08	0.64401
M	36	172	65	22	76	126	72	0.00915	16.125	0.12266
M	21	176	58	18.7	85	110	80	0.01583	12.9	0.07402
M	24	161	55	21.2	78	105	82	0.04929	14.798	0.2357
M	26	168	53	18.8	95	115	72	0.00468	21.25	0.27989
F	30	162	57	21.7	94	113	76	0.04307	11.375	-0.1048
M	32	179	62	19.3	89	108	80	0.02772	14.625	2.54445
M	21	175	65	21.2	75	107	90	0.03867	12.08	0.39909
M	20	161	55	21.2	88	102	75	0.00333	12.708	0.6697
M	24	192	70	19	79	100	85	0.05111	16.25	0.61318
M	20	172	57	19.3	95	119	70	0.00231	14.875	0.74814
F	36	170	60	20.8	90	116	80	0.0058	11.25	-0.6515
M	21	173	57	19	91	118	80	0.02916	10.7	-0.2918
F	27	169	62	21.7	97	106	90	0.0227	9.3	0.15657
F	20	172	52	17.6	89	109	85	0.0504	14.708	-2.293
F	20	164	48	17.8	98	103	78	0.0529	11.91	-0.1305
F	22	164	52	19.3	76	107	80	0.02681	69.16	0.10759
M	28	177	62	19.8	93	118	74	0.00398	15.04	-0.0884
M	20	173	55	18.4	78	112	79	0.00459	12.28	0.06883
F	24	168	57	20.2	90	110	72	0.01831	79.5	0.30352
M	32	164	60	22.3	94	100	80	0.02216	77.5	0.36963
M	20	171	58	19.8	75	108	90	0.00506	73.3	1.32704
F	20	164	49	18.2	86	117	70	0.03193	12.5	0.36415
M	29	172	59	19.9	68	106	85	0.03305	35	0.28023
M	28	173	52	17.4	89	112	75	0.03214	14.798	0.56077
F	20	162	47	17.9	90	116	73	0.00315	12.87	1.52298
F	27	159	45	17.8	87	114	72	0.02607	14.9	0.3597
M	23	172	56	18.9	85	101	76	0.01831	20.83	0.87792
F	22	167	55	19.7	91	105	75	0.00582	11.5	0.05244
F	25	158	58	23.2	99	118	72	0.04547	11.08	0.03771
M	36	164.23	65	24.1	95	104	78	0.04677	12.04	0.46417
M	40	173	68	22.7	83	108	80	0.03705	11.25	0.23844
F	28	169	57	20	72	111	90	0.02375	71.25	1.73364
M	22	174	56	18.5	94	120	86	0.05723	52.08	0.36381
M	24	169	59	20.7	90	110	80	0.02206	20.83	0.3114
F	40	164	55	20.4	92	115	80	0.04388	21.25	3.61113