

**“Dietary inflammatory index of north Karnataka
food pattern and its association with Coronary Artery
Diseases”**



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Medical Biochemistry
By
Dr. DEEPA S. SAJJANAR MBBS, MD

Ph.D. Research Scholar
Registration No: 15PHD001,

Department of Biochemistry,
Shri B. M. Patil Medical College, Hospital and Research Centre, BLDE
(Deemed to be University), Vijayapura, Karnataka, India

JUNE-2025



BLDE (DEEMED TO BE UNIVERSITY)

Shri B. M. Patil Medical College, Hospital and Research Centre Vijayapura,
Karnataka, India.

DECLARATION BY THE CANDIDATE

I hereby declare that this thesis entitled ***“Dietary inflammatory index of north Karnataka food pattern and its association with Coronary Artery Diseases”***

is bonafide and genuine research work carried out by me under the supervision of Dr. Indira A Hundekari, (Guide), Department of Biochemistry, Shri B. M. Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura, Karnataka, India and Dr. Rekha Udigiri, (Co-Guide), Department of Community Medicine and Dr. V G. Warad, (Co-Guide), Department of Medicine, Shri B. M. Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura. No part of this thesis has been formed the basis for the award of any degree, diploma, associateship, or fellowship, titles in this university or other similar institution of higher learning previously.

Signature of the Candidate

Dr. Deepa S. Sajjanar MBBS, MD

Date: **25-06-25**

Ph.D. Scholar

Registration No: 15PhD001 Department of Biochemistry,
Shri B. M. Patil Medical College, Hospital and Research Centre,
BLDE (Deemed to be University), Vijayapura, Karnataka, India



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Shri B. M. Patil Medical College, Hospital and Research Centre Vijayapura,
Karnataka, India.

CERTIFICATE BY THE GUIDE

This is to certify that this thesis entitled *“Dietary inflammatory index of north Karnataka food pattern and its association with Coronary Artery Diseases”* is a bonafide research work carried out by Dr. Deepa S.Sajjanar, under my supervision and guidance in the Department of Biochemistry, Shri B. M. Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura, Karnataka, India in the fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry.

Signature of the Guide

Dr. Indira A Hundekari,

Professor
Dept. of Biochemistry
BLDE (Deemed to be University),
Department of Biochemistry,
Shri B.M.Patil Medical College,
Vijayapur-586103,
BLDE (Deemed to be University),

Hospital and Research Centre,

Vijayapura, Karnataka, India.



BLDE (DEEMED TO BE UNIVERSITY)

**Shri B. M. Patil Medical College, Hospital and Research Centre Vijayapura,
Karnataka, India.**

CERTIFICATE BY THE CO-GUIDE

This is to certify that this thesis entitled *“Dietary inflammatory index of north Karnataka food pattern and its association with Coronary Artery Diseases”* is a bonafide research work carried out by Dr. Deepa S.Sajjanar, under our supervision and guidance in the Department of Biochemistry, Shri B. M. Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura, Karnataka, India in the fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry.

Dr V G Ward

Signature of the co- guide

Professor,

Professor of Medicine
Department of Medicine,
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre, Vijayapura
Hospital and Research Centre,
BLDE (Deemed to be niversity),
Vijayapura, Karnataka, India.

Dr. Rekha Ud giri

Signature of the co- guide

Professor and Head,

Prof. & HOD
Department of Community Medicine,
Dept. of Community Medicine
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
VIJAYAPURA-03.
Hospital and Research Centre,
BLDE (Deemed to be niversity),
Vijayapura, Karnataka, India.



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**Shri B. M. Patil Medical College, Hospital and Research Centre Vijayapura,
Karnataka, India.**

Endorsement by the Head of the Institution and Head of the Department

This is to certify that this thesis entitled “*Dietary inflammatory index of north Karnataka food pattern and its association with Coronary Artery Diseases*” is a bonafide research work carried out by **Dr. Deepa S. Sajjanar** under the supervision of **Dr. Indira A Hundekari**, (Guide), Professor, Department of Biochemistry, SBMPMC, Hospital and Research Centre, BLDE (D U), Vijayapura, Karnataka, India and **Dr. Rekha Udigiri**, (Co-Guide), Department of Community Medicine and **Dr. V G. Warad**, (Co-Guide), Department of Medicine, Shri B. M. Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University), Vijayapura, in fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry.

Signature of the HoD
Professor & HoD
Dept. of Biochemistry
BLDE (Deemed to be University)
Shri B. M. Patil Medical College,
Vijayapur-586103.
BLDE (DU) SBMPMC,
Hospital and Research Centre
Vijayapura, Karnataka, India

Signature of the Principal
Dr. Arvind V Patil
Principal,
BLDE (DU) SBMPMC,
Hospital and Research Centre
Vijayapura, Karnataka, India
PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & R.C. VIJAYAPURA-586103.



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**Shri B. M. Patil Medical College, Hospital and Research Centre Vijayapura,
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Dr. Deepa S. Sajjanar_{MBBS, MD}

PhD Scholar

Registration No: 15PhD001

Department of Biochemistry,

BLDE (DU) SBMPMC

Hospital and research centre

Vijayapur, 586103

Karnataka, India

Dedication

to my Family and Friends

And

*Special mention of my Husband,
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LIST OF ABBREVIATIONS

ACS – Acute Coronary Syndrome
ADA – American Diabetes Association
AGE -Advanced Glycation End-Product
AGE – Advanced Glycation End Product
AHA – American Heart Association
ANOVA (one-way analysis of variance)
BMI – Body Mass Index
BUN- Blood Urea Nitrogen
CAD – Coronary Artery Disease
CHD – Coronary Heart Disease
CI – Confidence Intervals
Cm – Centimeter
CRP – C-Reactive Protein
CVD – Cardiovascular Disease
DAG – Diacylglycerol
DALY (Disability-Adjusted Life Year)
DM – Diabetes Mellitus
ED – Endothelial Damage
EDGF – Endothelial Derived Growth Factor
EDTA – Ethylenediamine Tetra Acetic Acid
ECG - Electrocardiograms
ELISA - Enzyme Linked Immunosorbent Assay
FBG - Fasting Blood Glucose
Fe – Iron (implied by context as a nutrient)
FFA – Free Fatty Acid
GFR – Glomerular Function Test
Glucose Oxidase (GOD-POD)-Peroxidase
HbA1c – Glycosylated Hemoglobin
HDL-C – High Density Lipoprotein Cholesterol
High Performance Liquid Chromatography (HPLC)
HRP - Horseradish Peroxidase

hsCRP – High Sensitivity C Reactive Protein
IDDM – Insulin Dependent Diabetes Mellitus
IL – Interleukin
LDL-C – Low Density Lipoprotein Cholesterol
MACEs – Major Adverse Cardiac Events
MCP-1 – Monocyte Chemoattractant Protein-1
mg/dL – Milligram per Decilitre
Mg – Magnesium (implied by context as a nutrient)
mL – Milliliter
g – gram
m²-metersquare
mg-Milligram
mg/dl-milligramperdecilitre
mg/kg-milligramperkilogram
mIU/L-Milli International Units per Litre
mm³-cubicmillimetre
nm-Nanometre
mm of Hg – Millimetre of Mercury
MUFA – Monounsaturated Fatty Acids
n = number of sample
NADP – Nicotinamide Adenine Dinucleotide Phosphate
NCEP – National Cholesterol Education Programme
NF-κB – Nuclear Factor Kappa B
NIDDM – Non Insulin Dependent Diabetes Mellitus
NK Cell – Natural Killer Cell
NO – Nitric Oxide
NCD– Non-Communicable Diseases
NOS – Nitric Oxide Synthase
OD – Optical Density
OIES – Overall Inflammatory Effect Score
OR – Odds Ratios
Ox Lp(a) – Oxidised Lipoprotein (a)
PAI – Plasminogen Activator Inhibitor
PKC – Protein Kinase C

Plg – Plasminogen
PUFA – Polyunsaturated Fatty Acids
ROS – Reactive Oxygen Species
SD – Standard Deviation
SDMA – Symmetric Dimethyl Arginine
SMC – Smooth Muscle Cell
SNP – Single Nucleotide Polymorphism
SPSS – Statistical Package Social System
TC – Total Cholesterol
TF – Transcription Factor
TGF – Transforming Growth Factor
TG – Triglyceride
TNF- α – Tumor Necrosis Factor - α
tPA – Tissue Plasminogen Activator
VLDL – Very Low Density Lipoprotein
VSMC – Vascular Smooth Muscle Cell
WHR–Waist to Hip Ratio
WBC – White Blood Cell
WHO – World Health Organization
 μ IU/L – Micro International Units per Litre

ABSTRACT

Coronary artery disease (CAD) still remains as one of the chief causes of mortality and morbidity in the world. With a pooled prevalence of 11% noted for the young adult population in India, the statistics are intimidating. The major driving force in CAD is chronic, low grade systemic inflammation which is intrinsic to the activation, progression, plaque destabilization with eventual disruption of the coronary plaques leading to Major Adverse Cardiac Events (MACEs). The risk of CAD includes an intricate reciprocity between the genetic and the factors related to an individual's lifestyle. Of the many emerging risk factors, incessant inflammation known to be contributed by lifestyle and diet are important and are more than amenable to modification. The current study used the Dietary Inflammatory Index (DII®) to know the inflammatory potential of an individual's overall dietary intake as it is validated by numerous studies and against many inflammatory markers. Our study was aimed to investigate the association between DII, inflammatory biomarkers, CAD severity, and MACEs in a North Karnataka population with distinct dietary patterns.

Methods: This prospective cohort study was conducted over a period of two years and enrolled 310 CAD patients between the age of 18-76 year from district of Vijayapura, Karnataka, India. Dietary intake were calculated by the use of Food Frequency Questionnaire developed and validated particularly to be used in north Karnataka population. After calculating the DII scores, the CAD patients were divided into quartiles based on the DII scores. Q4 had CAD patients with higher DII scores which indicated the diet as proinflammatory and Q1 had CAD patients with lower scores whose dietary intake were considered anti-inflammatory. Baseline demographic, anthropometric, and clinical data of the CAD patients were noted and also Serum levels of high-sensitivity C-reactive protein (hsCRP), tumor necrosis factor-alpha (TNF- α), and interleukin-10 (IL-10) were estimated as markers of chronic systemic inflammation in them. The patients were followed for 30 days to document MACEs. The software SPSS 26 (SPSS Inc., version 0.21, Chicago, IL) was employed for the statistical analyses. Our study considered *p*-values less than 0.05 as statistically significant. The results were analysed using statistical analyses like ANOVA, Chi-square tests, and binary conditional logistic regression, and were adjusted for the many potential confounders of CAD such as age, gender, Body Mass

Index, smoking status, Hypertension, total serum cholesterol, Diabetes Mellitus(DM), physical inactivity to reduce the bias.

Results: The mean DII score of the CAD cohort was 2.28 ± 1.75 which indicated a dietary pattern which is predominantly pro-inflammatory in nature. A statistically significant association was observed between higher DII quartiles (Q4) and increased prevalence of older age ($p=0.01$), Hypertension status ($p=0.01$) and status of type-2 DM ($p=0.03$), and serum HsCRP levels showed an increase and was statistically significant ($p<0.001$) and the serum levels of IL-10 levels significantly decreased ($p<0.001$) with increasing DII quartiles (Q1 to Q4), suggesting a direct relationship between dietary inflammatory potential (DII scores) and systemic inflammatory load. An increased incidence of MACEs (20.6% overall) was significantly associated with higher DII scores ($p<0.001$), particularly second MI and urgent revascularization procedures. Unadjusted analysis showed a nearly 5-fold increased risk of MACEs in the highest DII quartile (OR=4.82, 95% CI: 2.2-10.6, $p<0.001$). The odds of MACEs in the highest DII quartile (Q4) remained 2.52 times higher than in the lowest (Q1) even after adjusting for known confounders

Conclusion: Diet with a higher DII score indicating a pro-inflammatory nature, is significantly correlated with increased systemic inflammation (higher hsCRP, lower IL-10) and a higher incidence of MACEs in CAD patients from North Karnataka. These findings underscore the crucial role inflammation associated with diet in the progression and adverse outcomes of CAD, highlighting the potential for dietary interventions as a personalized strategy for secondary prevention.

Keywords: Dietary Inflammatory Index (DII), Cardiovascular Disease (CVD) Outcomes, Chronic Inflammation, Precision Nutrition, Indian Population.

CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

Coronary artery disease (CAD) is one of the important cause of morbidity and mortality worldwide. About 9 million deaths are reported worldwide between 2007 and 2017 on account of ischemic heart disease and myocardial infarction as a consequence of this complex multifactorial disease(1). India is witnessing a rise in the CAD cases, with a pooled prevalence of 11% for the cardiovascular disease (CVD) noted for the young adult population, the statistics are intimidating (2). CVD are also the number one cause of death in India, accounting for a one-fourth of all cause mortality. The age-standardized CVD death rate among Indians is higher than the global average (3). The CAD that is documented in the Indian population is aggressive when compared with other East Asians and tends to affect the young working population which is a cause of increased concern (4), which is partly attributed to their newer acquisition of dietary patterns characterized by high intake of sodium, saturated and trans fats, (5) and recent lifestyle shift among them which is more obesogenic because of the economic and socio-cultural transitions (6). A wide regional variation is noted in DALY (Disability-Adjusted Life Year) rate due to CVD (14.1%) and is found to be highest in the states of Punjab and Tamil Nadu (7).

Apart from the premature onset and regional variations, poor management and high fatality rate are also of special concern in Indian population (8).The variations among different regions also extend to the reported cardio metabolic risk factors including psychosocial stress(9). The above concerns indicate the need for urgent policy and implementations in the health system which are deemed appropriate for each state (10).

The non-modifiable genetic factors and the lifestyle and environmental exposure during one's life period had an attributable rate of 21.6% and 78.4% respectively as reported in a Study by Rappaport et al. conducted on Swedish twins. (10).Thus, the risk of CAD includes an intricate reciprocity between the genetic and the factors related to an individual's lifestyle. It is also discerned that the CAD risk tends to be higher in individuals with a poor lifestyle with a genetic risk, low or otherwise and the highest risks of CAD are identified in individuals with a high genetic risk compounded by poor lifestyle (11). It has been touted by studies that a modest reduction of 35–60% in the mortality rate can be achieved by being perceptive about the CAD risk factors and drawing earnest measures aimed to prevent them at the

earliest(12).Over the years, efforts in the form of dedicated research were directed towards determining the cause for CAD. Such efforts have congregated ample evidence which support the fact that the major driving force in CAD is chronic, low grade systemic inflammation which is intrinsic to the activation, progression, plaque destabilization with eventual disruption of the coronary plaques as well(13).

The inflammation hypothesis also hold true for post-angioplasty, restenosis and for major adverse cardiac events (MACEs) reported in patients (14, 15).

The main mediators of chronic systemic inflammation noted are C-reactive protein, interleukin (IL)-1, IL-6, and IL-1 β , IL-18 among others. Other inflammatory markers like monocyte chemo attractant protein (MCP)-1, and tumor necrosis factor (TNF)- α and their expression also correlates with CAD severity and MACEs (16-18).

Research findings of the “inflammation hypothesis” paved way for the anti-inflammatory strategies which were further improvised employing the animal models. Interventions are also being targeted at every step of or the factors linked with the inflammation. The success of two of the obtrusive clinical trials in this direction- The RESCUE-2 trial (19) and Colchicine Cardiovascular Outcomes Trial (COLCOT) (20) have reinforced the *avant -garde* support to the anti-inflammatory hypothesis of atherosclerosis and also can help achieve a more precise and personalized therapy(21) for the secondary prevention in CVD patients. Despite the fact that many of the approaches against the inflammation were inadequate to date in preventing the atherosclerotic CVD, considerable efforts are continued to be directed against it. One such understanding is the certainty that Systemic inflammation and oxidative stress are more than ever amenable to modification by nutrition (22-24).

Asseveration to which can be noted when we look at the dietary patterns of "Blue Zones," which emphasizes a plant-based, antioxidant-rich diet which possibly reduces inflammation and oxidative stress, contributing to longevity and low rates of chronic diseases including a favorable cardiovascular health (25). Diet can modulate inflammation relevant to CVD possibly through significant reductions in CVD risk factors like blood pressure and inflammatory markers (26).

Some of the established dietary patterns like the western dietary pattern known to include refined, ultraprocessed and high saturated fats tend to activate excessive

production of pro-inflammatory cytokines and also a concomitant reduction of the synthesis of anti-inflammatory cytokines (27-29).

In contrast, the Mediterranean diet (MeDiet) which is touted to be the most studied diets for CVDs has shown to lower the load and even impede the ontogenesis of CVD. An improvement in the CVD predictors such as the lipid levels, waist-to-hip ratio and inflammatory markers are recognized. Data from studies, both observational and randomized controlled trials on primary outcomes in those consuming an anti-inflammatory diet have reported a decrease in death and clinical events due to CVD (30-33).

MeDiet and DASH (Diet Against Stopping Hypertension) diet can also be cardioprotective for the fact that they do positively affect the composition and activity of gut microbiota and cause a consequent reduction in the Trimethylamine N-oxide (TMAO) levels.

TMAO is a metabolic product produced by the action of gut bacteria on dietary components like L-carnitine, betaine, and choline. Elevated TMAO levels can cause vascular injury and increased risk of DM, hypertension and atherosclerosis (34).

Hence, dietary modifications appear to be the best target in the snowballing challenge against CVD as they can easily emulate the well established measures such as aspirin, beta-blockers, ACE-inhibitors, and physical activity (30).

The dietary modifications are lauded to be an important component of the lifestyle recommendations for the population at large (35-36), educational institutions and health providers throughout the world should play a more responsible role in identifying and propagating food system solutions to unhealthy diets and also advocate communication for behavioral change (37).

Hence, when inflammatory properties are used to classify the dietary intake of individuals the findings can yield important information about the links between diet, inflammation, and CVD (38) which should be explored scientifically. Dietary-inflammatory index (DII[®]) was contrived by Shivappa and his colleagues after years of research in this direction. DII is a literature-derived dietary tool which is based on the capacity of diets to modulate systemic inflammatory biomarkers (39) and stands validated with several inflammatory markers (40-41).

The Dietary Inflammatory Index (DII) is based on the dietary analysis of the principal components and categorizes individual's diet as anti- or pro-inflammatory. It can be a great practical tool for prevention of coronary artery disease (CAD) and MACEs (42). Preliminary studies, investigating the association between the DII and risk of CVD have reported that for each 1-unit increase of the DII score, the risk of incident CVD and the associated mortality increases by a substantial 8% (43).

Despite decoding the most advantageous dietary patterns for CVD prevention, significant knowledge gaps do prevail to date. The need of the present study is to document any evidence for the association of DII with the disease severity, its progression and the inflammatory load in the CAD patients which shall be thoroughly inquired and documented in the regional population of north Karnataka which has a distinct dietary pattern. The study findings will further help us derive a more robust dietary risk assessment and modification, one which is evidenced based and acceptable with ease by the locals. It will also add immense value in our efforts to develop dietary interventional strategies in preventing cardiac events at large. It can also be a useful tool for promoting clinical and public health by conducting counseling and educational activities on health and nutrition.

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CHAPTER 2

AIM AND OBJECTIVES

OF THE STUDY

2.1 – Aims and objectives

Aim: To determine the DII of the CAD patients primarily consuming north Karnataka food pattern and to document and assess the occurrence of Major adverse cardiac events with disparate DII scores.

Objectives:

1. To determine and document the dietary inflammatory index of Coronary Artery Disease patients, primarily consuming the north Karnataka food pattern.
2. To document the MACEs in patients with disparate DII scores and to assess the association between DII, serum levels of inflammatory markers and MACEs.

Hypothesis

Null hypothesis:

The major adverse cardiac events in CAD patients do not occur more often in those with higher DII scores and may not be reflected as higher inflammatory load.

Study hypothesis:

The major adverse cardiac events in CAD patients occur more often in those with higher DII scores and is reflected as higher inflammatory load.

CHAPTER 3

REVIEW OF

LITERATURE

3.1 Coronary Artery Diseases (CAD)

Coronary heart disease or Coronary Artery Disease (CAD) occurs due to atherosclerosis causing narrowing and/or blocks in the coronary arteries leading to reduced blood flow to the heart muscle. The reduced flow in the coronary vessels causes chest pain (angina), heart attack, or heart failure (1).

3.1.1 Epidemiology and Prevalence of CAD

CVDs constitute a formidable global health challenge, consistently ranking as the leading cause of mortality and morbidity worldwide (1). In 2021, these conditions were responsible for approximately one-third of all global deaths (2). About three-quarters of CVD-related fatalities are documented in India along with the other low- and middle-income countries (LMICs) (3).

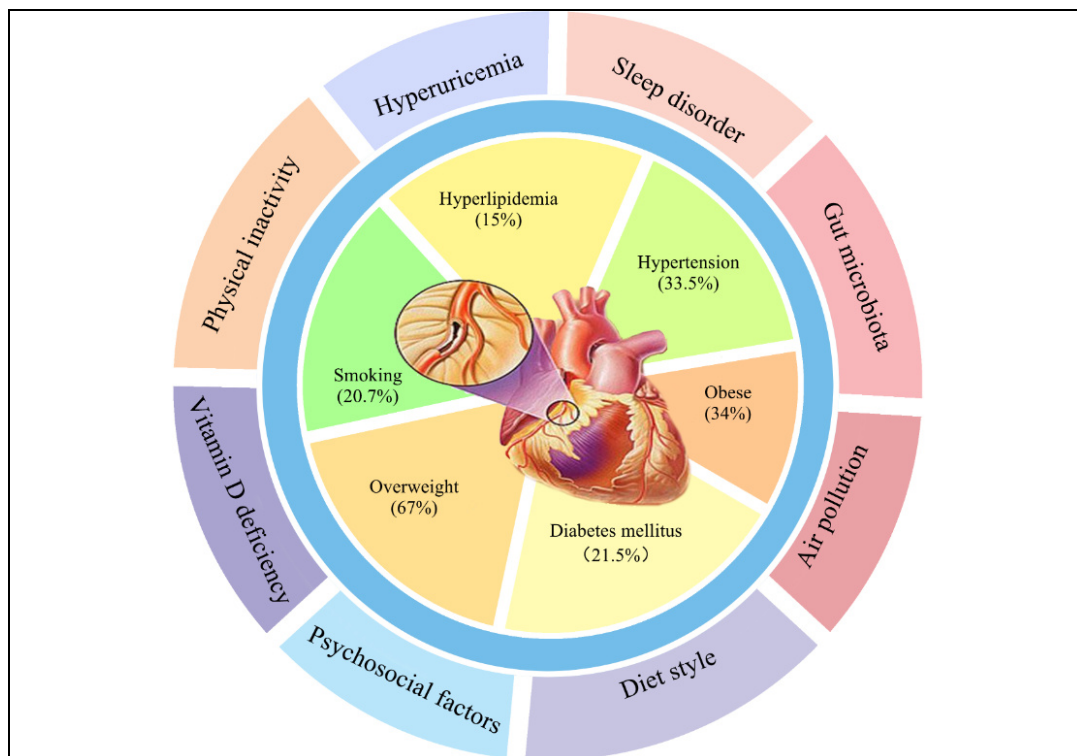
Of the one-fifth of all global CVD-related deaths occurring in India, particular concern is the impact observed within its younger demographic (2). The global average of age-standardized CVD death rate was 235 per 100,000 in 2019 and India reported the same at 272 per 100,000 population (4). Furthermore, the documentation of 32,457 heart attack deaths in 2022 alone underscores coronary artery disease (CAD) as a persistent and escalating public health challenge within the nation (5). Analysis of CVD prevalence reveals a pooled rate of 11% across India. A slightly higher prevalence is observed in females (14%) compared to males (12%) (6). Consistent with the global trend, a more pronounced disparity exists between urban and rural areas, with significantly higher prevalence in urban settings (12%) versus rural areas (6%). This discrepancy is intrinsically linked to urbanization-associated lifestyle modifications, such as altered dietary habits and decreased physical activity.

These behavioural changes contribute to elevated rates of obesity, hypertension, and diabetes, which are established major risk factors for CVD. (7) This pronounced urban-rural disparity highlights an escalating public health crisis within the country. A more comprehensive understanding of these contributing factors is crucial for facilitating early CVD detection and informing the development of effective prevention strategies to address this growing health challenge.

3.1.2 Risk Factors for CAD (8)

CAD is a multifactorial disease affected by both non-modifiable and modifiable risk factors. Recent research has further elucidated the complex mechanisms underlying its development and progression. While traditional risk factors remain central, recent scientific evidence highlights a more intricate interplay of genetic, environmental, and behavioral factors. Here's an updated perspective on key risk factors for CAD.

Figure3.1: Classification of risk factors for Coronary artery disease (CAD)



The traditional CVD factors (First circle), and the new non-traditional (second circle) CVD risk factors.

A) Traditional Modifiable Risk Factors:

- i) **Dyslipidaemia:** Dyslipidaemia, characterized by aberrant concentrations of circulating lipoproteins, constitutes a formidable and independent risk factor for the initiation as well as progression of coronary artery disease (CAD). LDL cholesterol has an important role in atherogenesis and can be considered as causal, serving as the primary therapeutic objective in lipid-lowering interventions. Elevated levels of triglyceride-rich lipoproteins (TRLs) and their remnants are now recognized as direct contributors to vascular pathology, promoting inflammation and endothelial dysfunction. Furthermore, increased lipoprotein(a) (Lp (a)) concentrations, largely genetically determined, confer a substantial and independent risk for premature CAD, irrespective of other lipid parameters, highlighting its unique atherogenic and prothrombotic properties. The pathological mechanisms linking dyslipidemia to CAD involve a complex cascade of events, including chronic vascular inflammation, oxidative stress, and the direct deposition of lipids within the arterial intima. Given the compelling evidence, current clinical guidelines advocate for aggressive lipid-lowering regimens, primarily employing statins, to achieve stringent LDL-C targets (9,10).
- ii) **Hypertension:** Hypertension represents a predominant and an independent causal factor for the risk of development and exacerbation of CAD. Sustained elevations in systemic blood pressure directly initiate and perpetuate atherosclerotic processes through inducing endothelial injury, promoting vascular inflammation, increasing oxidative stress, and altering arterial wall mechanics. These pathological changes facilitate the genesis and progression of coronary plaque formation. Furthermore, chronic hypertension engenders adverse cardiac remodeling, notably left ventricular hypertrophy, which compromises myocardial oxygen supply-demand balance and microvascular function, thereby augmenting ischemic susceptibility. The resultant increase in arterial stiffness and pulse pressure serves as an additional independent prognostic indicator for CAD events. Contemporary clinical guidelines advocate for stringent blood pressure control as robust evidence demonstrates that effective antihypertensive therapy profoundly attenuates the incidence of myocardial infarction and other adverse cardiovascular outcomes (11,12).
- iii) **Diabetes Mellitus:** As one of the profound and independent risk factor for the development as well as an accelerated progression of CAD, diabetics demonstrate a markedly elevated propensity for atherosclerotic cardiovascular disease (ASCVD),

characterized by a more widespread and complex coronary atherosclerosis burden. The underlying pathophysiology is multifactorial, involving chronic hyperglycemia-induced endothelial dysfunction, exacerbated oxidative stress, a sustained pro-inflammatory state, and the formation of advanced glycation end products.

These mechanisms collectively promote the initiation and growth of unstable atherosclerotic plaques. Furthermore, diabetes is frequently accompanied by an atherogenic dyslipidemia and a heightened prothrombotic propensity, all contributing significantly to CAD risk.

The frequent co-occurrence of diabetes with other Key contributors to heart and blood vessel disease, such as hypertension and obesity, results in a synergistic amplification of atherogenic processes. Recent focus is on how newer anti-diabetic medications, such as drugs acting to inhibit SGLT2 receptors and agonists of GLP-1 receptors, provide cardiovascular advantages beyond their primary role in blood sugar control (13,14).

- iv) Obesity: Increased visceral adipose tissue now regarded as an endocrine organ in itself is a potent risk factor for CAD. It increases systemic inflammation, endothelial dysfunction, insulin resistance, sympathetic nervous system activation, increased vascular resistance, and a prothrombotic state, all of which are central to the pathogenesis of atherosclerosis and CAD. These interconnected pathways collectively promote the initiation and advancement of atherosclerotic plaque formation. The accumulation of visceral and ectopic fat depots (e.g., epicardial fat, liver fat) are particularly strong independent predictors of adverse cardiovascular outcomes as well. Clinically significant weight reduction, achieved through comprehensive lifestyle interventions, pharmacotherapy, or bariatric surgery, has demonstrated profound improvements in cardio metabolic risk profiles. A reduction in the incidence of major adverse cardiovascular events is also seen underscoring the critical importance of obesity management in CAD prevention and treatment strategies. (15,16)
- v) Smoking: Tobacco use, including traditional cigarettes, e-cigarettes, and smokeless tobacco, is still one of the most potent modifiable risk factors. It directly damages the endothelium and is known to promote thrombosis and inflammation. Additionally, smoking leads to coronary occlusion by exposing and baring the endothelium, causing increased adhesion of platelets to the sub intimal layers, augmenting infiltration of lipids, and proliferation of smooth muscle cells which is stimulated by platelet-

derived growth factor (PDGF). An estimated 30–40% of annual deaths are associated with CAD and attributed to smoking (17).

- vi) Consistent findings from both the cohort study by Benjamin, E. J., et al. (18) as well as case–control study by Bhatnagar, A., et al. (19) on over twenty million individuals highlight a heightened incidence of CAD-related deaths among smokers in comparison to non-smokers. The deleterious effects of cigarette smoking on cardiovascular disease demonstrates a dose–response relationship. The duration of smoking, the number of cigarettes and also the depth of smoke inhalation can determine the risk of developing CAD. These studies reinforce the immediate and long-term benefits of smoking cessation across all age groups.
- vii) Physical Inactivity and Unhealthy Diet: Sedentary lifestyles and diets high in saturated fats, trans fats, processed carbohydrates, and sodium continue to drive the global burden of CVD. Current research emphasizes the role of personalized nutrition and active lifestyle interventions in preventing and managing CAD (20,21).

B) Emerging and Non-Traditional Risk Factors:

- a) Chronic Inflammation: Chronic systemic inflammation is now unequivocally recognized as a pivotal and causal risk factor in the pathogenesis as well as progression of CAD. All stages of atherosclerosis, from initial endothelial dysfunction and lipid infiltration to the eventual destabilization and rupture of atherosclerotic plaques are influenced by inflammation. Beyond lipid deposition, the inflammatory milieu within the arterial wall dictates plaque vulnerability, primarily by promoting fibrous cap degradation and extracellular matrix remodeling, thereby increasing the propensity for thrombotic events. Key inflammatory mediators, mainly the C-reactive protein (CRP) and interleukins like IL-1 β , IL-6, serve as critical biomarkers and direct contributors to this process.

A significant contemporary understanding is the intricate crosstalk between metabolic dysregulation and inflammatory pathways; for instance, conditions such as obesity and dyslipidemia actively fuel systemic inflammation, thereby accelerating atherogenesis. The appreciation of inflammation's central role has paved the way for novel therapeutic strategies, with clinical trials demonstrating that targeted anti-inflammatory interventions can independently reduce major adverse cardiovascular events, even in patients with well-controlled lipid levels (22,23).

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- b) **Genetic Predisposition:** Genetic predisposition contributes substantially to an individual's susceptibility to CAD, with the estimates of influence ranging from 40% to 60%. This inherited risk is primarily polygenic, stemming from the cumulative effect of numerous common single nucleotide polymorphisms (SNPs), each conferring a modest individual effect. Polygenic risk scores (PRS) integrate information from these myriad genetic variants, providing a comprehensive, birth-quantifiable metric of an individual's inherent CAD risk, independent of established modifiable factors. Genome-wide association studies have not only corroborated known associations but also elucidated novel biological pathways implicated in atherogenesis. Genetic risk can be significantly modulated by lifestyle and environmental factors. Individuals with a high genetic risk can substantially reduce their CAD incidence by adopting a healthy diet, regular exercise and cessation of smoking which constitutes a healthy lifestyle. Advanced genetic risk assessment facilitates the implementation of personalized preventive strategies, enabling targeted and potentially earlier interventions to optimize cardiovascular outcomes.(24,25)
- c) **Gut Microbiome Dysbiosis:** Gut dysbiosis is an alteration in the equilibrium of the intestinal microbiota which is increasingly being recognized as an important independent risk factor for CAD. A primary mechanistic link involves elevated circulating TMAO levels that demonstrably promote atherogenesis by inducing endothelial dysfunction, fostering a pro-inflammatory state, enhancing platelet hyperactivity, and perturbing cholesterol metabolism.
- Furthermore, gut dysbiosis can compromise intestinal barrier integrity, leading to the translocation of bacterial components (e.g., lipopolysaccharides) into the systemic circulation, thereby initiating or exacerbating chronic low-grade inflammation that drives atherosclerotic progression. The gut microbiome also critically modulates host lipid and glucose homeostasis mainly by its action on bile acids metabolism and the production of fatty acids with of shorter chains or SCFAs. The reduction in beneficial SCFA-producing bacteria is being associated with adverse cardiovascular profiles. Consequently, interventions aimed at rectifying gut dysbiosis, including dietary adjustments, prebiotics, probiotics, and targeted inhibitors of microbial pathways, represent promising therapeutic avenues for the prevention and management of CAD (26,27).

Other emerging risk factors include sleep disorders (28), air pollution (29), Psychosocial Stress (30) and Mental Health (31) and also Socioeconomic Disparities and Social Determinants of Health (32).

A comprehensive understanding of these traditional and emerging risk factors is vital for developing targeted prevention strategies, particularly in regions like India, where the burden of CAD continues to escalate. Addressing these multifaceted determinants, from individual behaviours to broader environmental and social contexts, is paramount to mitigating the pervasive impact of CAD.

3.1.3.1 The inflammatory process in CAD

Atherosclerosis is an inflammatory disease of chronic nature which affects blood vessels which is primarily initiated by the accumulation of LDL-C. Blood flow within the vessel lumen is hindered by the development of subintimal plaques which are complex in nature. This eventually increases the risk of rupture or erosion of the plaque ultimately causing thrombotic occlusion and tissue infarction.

Among the initial processes driving atherosclerosis is the endothelial denudation exposing it to various harmful stimuli, including hypertension, hypercholesterolemia and elevated pro-inflammatory cytokines. These factors interfere with the endothelium's normal role as a protective barrier, causing it to become activated. When activated, the uptake of lipoproteins from the bloodstream increases, notably of those containing ApoB and LDL-C. Subsequently, these lipoproteins interact with sub endothelial proteoglycans in the extracellular matrix and are retained in the arterial wall. Interactions with the pro-oxidative enzymes, locally released reactive oxygen species (ROS), and lipid oxygenases produce diverse chemical modifications in them, important one being the oxidation of the lipoproteins. (33) Endothelial cells on Activation upregulate important leukocyte adhesion molecules like Vascular Cell Adhesion Molecule-1 (VCAM-1) and also the Intercellular Adhesion Molecule-1 (ICAM-1) (34) facilitating leukocyte recruitment to the vessel wall, especially neutrophils and monocytes.

Initially, E-selectin and P-selectin which are the selectin-dependent adhesion molecules enable leukocyte adhesion, followed by firmer attachments via VLA-4 binding to VCAM-1 and ICAM-1. Animal studies show reduced lesion size in mice knocked out for ICAM-1 and also P-selectin and those lacking VCAM-1 expression (35).

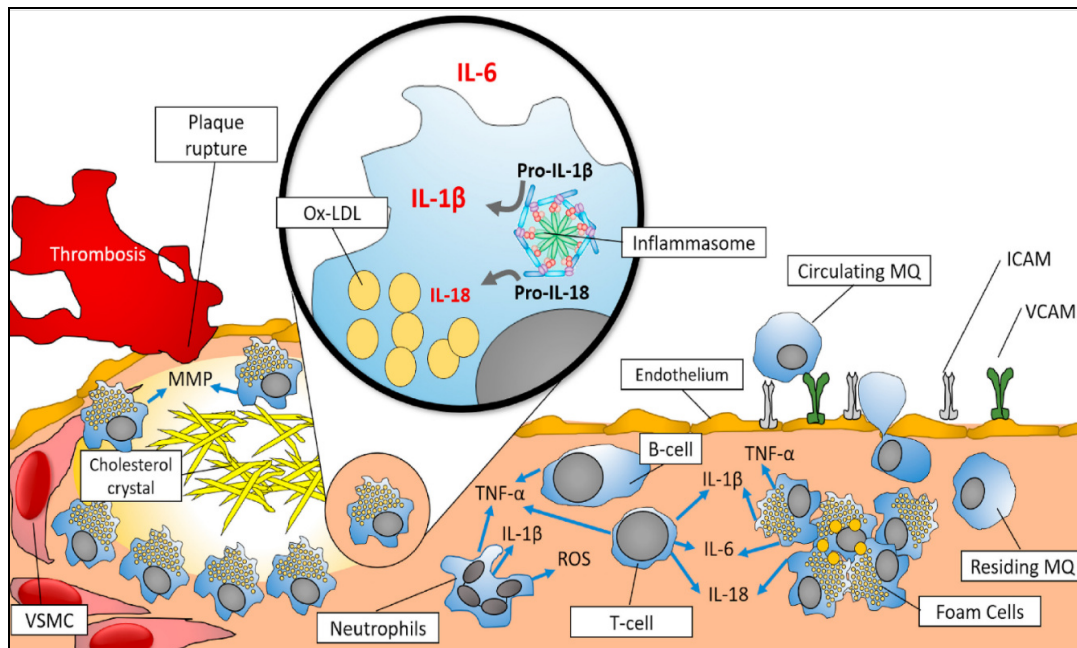
Growth factors like M-CSF stimulate the monocytes to differentiate into macrophages in the arterial wall. Macrophages phagocytose modified LDL-C particles, becoming foam cells which are lipid-filled, causing yellowish fatty streaks in the vessel wall, indicating early atherosclerotic disease (36). Macrophages uptake the oxLDL mainly via scavenger receptors (SRs), notably CD-36 and SR-A1, contributing to about 90% of oxLDL uptake (37). Initially, this oxLDL uptake serves a protective function by sequestering potentially harmful lipoproteins. However, with time, foam cells become overwhelmed in their capacity to metabolise these lipoproteins. An apoptotic process is initiated due to the stress in endoplasmic reticulum caused by the generation of reactive oxygen species (ROS) (38).

3.1.3.2 Biomarkers of Inflammation in CAD

The progression of atherosclerotic plaques, from an initial fatty streak to a more complex fibrous lesion triggers the release of various pro-inflammatory cytokines which further increases the infiltration of immune cells and aggravates atherosclerosis. Additionally, ineffective clearance of dead cell components via efferocytosis can lead to the build-up of necrotic debris. This situation predisposes the plaque to develop a necrotic lipid-rich core. Thus the plaques becomes highly thrombogenic (39). Degradation of collagen and subsequent thinning of the fibrous cap have been recognized as significant factors contributing to plaque rupture or erosion, leading to myocardial infarction, unstable angina and sudden cardiac death termed as Major adverse cardiac events (MACEs) and constitute as acute coronary syndromes.

Atherosclerotic progression is critically dependent on the migration and phenotypic alteration of vascular smooth muscle cells (SMCs) which is influenced by these pro-inflammatory cytokines. SMCs in the intima then proliferate and primarily synthesize copious amounts of types 1 and 3 collagen proteins among other extracellular matrix (ECM) proteins and proteoglycans. This extensive ECM production by activated SMCs is pivotal for the remodeling and stabilization of the plaque, directly contributing to the progression of CAD. In essence, pro-inflammatory cytokines are key instigators in driving SMC proliferation and subsequent plaque development in CAD (40).

Figure 3.2: Role of inflammation in atherosclerosis (40)



Apart from macrophages, T and B lymphocytes also play significant role in atherosclerosis. Despite their smaller numbers, T cells, appear early in plaques and modulate the inflammatory response initiated by macrophages. T cells have diverse functions; Th-1 cells produce pro-inflammatory cytokines like $\text{TNF-}\alpha$, $\text{IFN-}\gamma$ and IL-2 which promote atherosclerosis (41). While T-reg cells secrete IL-10 and $\text{TGF-}\beta$ which are considered as anti-inflammatory cytokines (42). B cell functionality depends on subtype; B1 cells may protect against atherosclerosis by generating antibodies to atherogenic antigens (43), whereas B2 cells can exacerbate atherogenesis by secreting pro-inflammatory cytokines and activating Th-1 cells (44). Experimental models by Ramji, D. P. et al, show a 40% to 80% reduction in atherosclerosis burden in the experimental models lacking T and B cells (45) suggesting their significant role. The overall impact Of CAD is likely more determined by the balance between their pro-inflammatory and anti-inflammatory subsets

It is crucial to emphasize that inflammation is no longer considered a mere bystander in atherosclerosis, but a central, active participant in its initiation, progression, and ultimately, its thrombotic complications and acts as a general predictor of adverse outcomes (40) and further research is necessary to understand their role in CAD development and progression.

3.1.3.3 Key Inflammatory Markers in CAD Pathogenesis and Prognosis

Figure 3.3: Key inflammatory markers in Coronary artery disease.

Marker	Type of Molecule	Role in CAD and Atherosclerosis
C-Reactive Protein (CRP)	Acute-phase protein	- Central mediator of acute phase response.
Interleukin-6 (IL-6)	Cytokine: Pro-inflammatory	- Drives the acute phase response, stimulating CRP
TNF-alpha	Cytokine: Pro-inflammatory	- Key mediator in inflammatory and immune response
Myeloperoxidase (MPO)	Leukocyte-derived enzyme	- Directly involved in oxidative stress and inflammation
Soluble CD40 Ligand (sCD40L)	Adhesion molecule, shed from cell surface	- Involved in immune response regulation and inflammation
Pentraxin 3 (PTX3)	Long pentraxin	- An important component of the innate immune system
Matrix Metalloproteinases (MMPs)	Enzymes	- Involved in the degradation and remodeling of the fibrous cap
Fibrinogen	Coagulation factor, acute-phase protein	- Key component in blood coagulation and function
Serum Amyloid A (SAA)	An acute-phase protein	- Present in HDL in plasma.
Adhesion Molecules (e.g., sICAM-1, sVCAM-1,	Cell surface proteins (soluble forms)	- Mediate leukocyte adhesion to endothelial cells.
Galectin-3	Lectin protein	- Involved in inflammation, fibrosis, and cell growth.
Lipoprotein-associated Phospholipase A2 (Lp-PLA)	Enzyme	- Hydrolyzes oxidized phospholipids in LDL.

3.1.3.3 a Pro-inflammatory Cytokines: Role in CAD Risk, Severity, and Outcomes

i) High-Sensitivity C-Reactive Protein (hsCRP)

Among the plethora of the inflammatory biomarkers investigated, C-reactive protein (CRP) was one of the earliest marker to have attracted considerable attention and is implicitly recognized as a valuable prognostic tool for recognising individuals at increased risk for any cardiovascular events in future, even if the overt clinical symptoms are absent (46,47).

A recent meta-analysis by Peng J et.al, has concluded that elevated baseline C-reactive protein level remains an important and independent predictor of MACEs in patients with stable CAD. The adjusted risk ratio (multivariable) for MACEs was 1.77 with 95% CI between 1.60–1.96 when comparing the highest to the lowest CRP levels. This indicates that individuals with higher CRP levels are significantly more likely to experience the MACEs. They also found a strong association between elevated baseline CRP and both cardiovascular mortality with a pooled risk ratio of 1.64 and 95% Confidence Interval between 1.13–2.33 and a pooled risk ratio 1.62 for all-cause mortality. This underscores CRP's ability to identify patients at higher risk of death regardless of the cause. Additionally, elevated CRP levels do likely reflect a "residual inflammatory risk" that is not fully addressed by traditional risk factor management (48).

A notable investigation conducted by Sabatine, M. S et.al, on the data derived from the PEACE trial, involved 3,771 patients with stable CAD who were followed up for a 4.8 median years. They found that the elevated hs-CRP concentrations significantly correlated with an higher risk of MACEs. Specifically, the hs-CRP levels (1 -3 mg/L) were associated with an adjusted hazard ratio (HR) of 1.39 with the 95% Confidence Interval between 1.06 and 1.81; with a 'p' value=0.016, while levels more than 3 mg/L showed a higher adjusted HR of 1.52 and more significant risk for MACEs with p=0.003. These findings have subsequently spurred interest in utilizing hs-CRP to guide therapeutic strategies in stable CAD (49).

ii) Tumor Necrosis Factor-alpha and IL-6

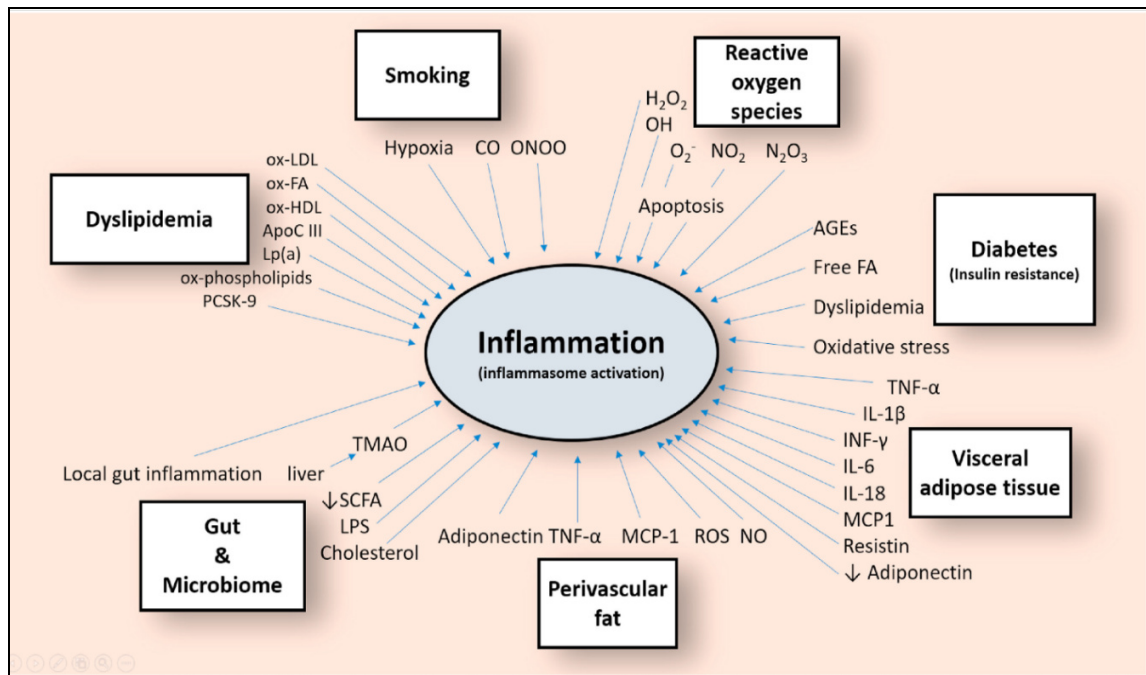
Chronic low-grade inflammation is now unequivocally recognized as a pivotal driver in the initiation, progression, and acute complications of CAD. Central to this inflammatory cascade are Tumor Necrosis Factor-alpha (TNF- α) and Interleukin-6 (IL-6) which are considered as the key pro-inflammatory cytokines.

These potent mediators orchestrate a series of detrimental events within the vascular endothelium and atherosclerotic plaque, profoundly impacting CAD progression and the risk of MACEs and mortality.

In addition to hsCRP, Del Giudice, M et.al, have explored interleukin 6 (IL-6), an inflammatory mediator, as a potential diagnostic marker for obstructive CAD. IL-6 levels have been found to rise with age and in individuals with a higher BMI (50).

Coronary angiography in 48 patients referred for non-acute chest pain were included in a small cross-sectional study, patients with IL-6 levels >1.0 pg/mL exhibited a positive predictive value (PPV) for obstructive CAD of 100% and IL-6 had a better predictive value than the hsCRP levels (51). IL-6 is also known to stimulate the production of C-reactive protein (CRP) and other inflammatory mediators. Elevated IL-6 levels are strongly linked to increased CAD risk, plaque formation, and vulnerability (52) along with TNF- α (53) which can independently predict a significantly higher risk (~3-fold increase in risk) of MACEs (54,55). The NLRP3 (Nucleotide-binding oligomerization domain-like receptor protein (40) is an important component of the innate immune system that plays a central role in inflammation. NLRP3 inflammasome along with its downstream cytokines (IL-1 β and IL-18 directly and IL-6 and TNF- α , indirectly) are central to sterile inflammation in atherosclerosis and elevated inflammatory markers, highlight the direct link between this pathway and MACE. Targeting these pathways significantly reduces recurrent cardiovascular events in patients with prior MI (56).

Figure 3.4: NLRP3 inflammasome activation and the various cytokines (56)



Role of the pro-inflammatory markers in CAD pathogenesis:

- Macrophage Migration Inhibitory Factor (MIF), a multi-functional cytokine that is known to counteract the anti-inflammatory effects of glucocorticoids and amplifies inflammatory responses (71) and also

MicroRNAs (miRNAs) such as miR-155, miR-146a, miR-133a which are small non-coding RNAs which regulate gene expression in inflammatory pathways and stand dysregulated in CAD.(72) The pathogenesis and progression of CAD is thus proved to be driven **a) Endothelial Dysfunction:** The endothelium is a crucial interface regulating vascular tone, permeability, and immune cell interactions. In the context of CAD, TNF- α and IL-6 are significant contributors to endothelial dysfunction, an early and critical step in atherogenesis. TNF- α directly impairs nitric oxide (NO) bioavailability, a key vasodilator and anti-atherogenic molecule, by increasing oxidative stress and upregulating arginase activity. The arginase tends to compete with endothelial nitric oxide synthase (eNOS) for the substrate (57).

Both TNF- α and IL-6 induce the expression of VCAM- and ICAM-1 (58). This up regulation transforms the normally non-adhesive endothelium into a pro-adhesive surface, setting the stage for immune cell infiltration.

b) Leukocyte Recruitment: The heightened expression of adhesion molecules, driven by TNF- α and IL-6, facilitates the recruitment of leukocytes, particularly monocytes, from the bloodstream to the arterial intima. These circulating monocytes adhere to the activated endothelium and subsequently transmute into the subendothelial space. Once within the intima, they differentiate into macrophages, which are central players in atherosclerotic plaque development. This continuous influx of inflammatory cells, largely orchestrated by these cytokines, sustains the inflammatory process within the vessel wall (59).

c) Plaque Destabilization: Beyond their role in early atherogenesis, TNF- α and IL-6 are critically involved in plaque destabilization, the process by which a stable atherosclerotic lesion becomes prone to rupture, leading to acute coronary syndromes. Within the plaque, macrophages, activated by TNF- α and IL-6, release a plethora of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases. The MMP-2 and MMP-9 cause the degradation of the extracellular matrix components, such as collagen and elastin, which provide structural integrity to the fibrous cap of the plaque.

This enzymatic degradation thins and weakens the fibrous cap, making the plaque vulnerable to rupture. Furthermore, TNF- α can induce apoptosis of Vascular Smooth Muscle Cells within the fibrous cap, further compromising its stability (60). IL-6 also promotes the proliferation of VSMCs, which, depending on the context, can contribute to both plaque growth and, paradoxically, plaque instability if the proliferation is disorganized (58).

Association of inflammatory markers with CAD Progression and Increased Risk of MACEs and Mortality:

Inflammation is involved in every stage of atherosclerosis, from endothelial dysfunction to foam cell formation, plaque development, progression, and ultimately, plaque rupture and a significantly increased risk of MACEs and mortality. Higher baseline levels of these cytokines predict can predict the future cardiovascular events. (58, 52) The sustained pro-inflammatory environment fostered by these cytokines contributes to continuous endothelial damage, ongoing plaque growth, and the heightened likelihood of plaque rupture and subsequent thrombotic events. In patients with acute coronary syndromes (ACS), elevated TNF- α and IL-6 levels at presentation are associated with impaired endothelial function and a higher risk of MACEs during follow-up, underscoring their prognostic utility (54). Therefore, TNF- α and IL-6 are

not merely markers of inflammation but active participants in the pathological processes that drive CAD and its devastating clinical consequences.

3.1.3.3b Anti-inflammatory Cytokines: Interleukin-10 (IL-10) and its Protective

The balance between the pro-inflammatory and anti-inflammatory cytokines may offer a more nuanced and powerful prognostic indicator than individual cytokine levels alone.

Role of the Anti-inflammatory markers in CAD pathogenesis:

Interleukin-10 (IL-10), produced predominantly by regulatory T cells (Tregs), macrophages, and B cells stands as a pivotal anti-inflammatory cytokine with a crucial protective role in coronary artery disease (CAD). Whereas, an elevated TNF- α /IL-10 ratio, for instance, has been correlated with increased inflammatory response in CAD patients (60). It acts as an endogenous brake on excessive immune responses, thereby mitigating the chronic inflammation central to CAD pathogenesis.

- **Immune modulation and cytokine suppression:** At a fundamental level, IL-10 dampens the activation of immune cells, particularly monocytes and macrophages. It achieves this by directly suppressing the activation of key transcription factors, most notably **NF- β** , which is essential for the expression of pro-inflammatory genes (61, 62). This inhibition leads to a significant reduction in the synthesis and release of detrimental cytokines like TNF- α , IL-6, and IL-1 β , effectively neutralizing the inflammatory milieu that drives plaque progression and instability (58,62). Beyond direct cytokine suppression, IL-10 modulates immune responses by:
- **Inhibiting antigen presentation:** The expression of Major Histocompatibility Complex (MHC) class II molecules is reduced by IL-10. It can also reduce the expression of co-stimulatory molecules on Antigen-Presenting Cells (APCs) thereby limiting T-cell activation and proliferation.
- **Reducing oxidative stress and foam cell formation:** The production of reactive oxygen species (ROS) by macrophages is reduced by IL-10 which in turn limits LDL oxidation and subsequent foam cell formation (62).
- **Suppressing matrix metalloproteinase (MMP) activity:** By inhibiting the production of MMPs and promoting the expression of tissue inhibitors of metalloproteinases (TIMPs) from macrophages, IL-10 helps preserve the integrity of the fibrous cap, thereby reducing the likelihood of plaque rupture (62).

-
- **Promoting anti-inflammatory macrophage phenotypes:** IL-10 encourages the polarization of macrophages towards an M2 (anti-inflammatory) phenotype, which is characterized by efferocytosis - clears the apoptotic cells, tissue repair, and synthesis of anti-inflammatory mediators. This contrasts with the M1 (pro-inflammatory) phenotype that drives plaque progression (63).

Mallat Z,et.al, have demonstrated that IL-10 deficiency significantly exacerbates plaque formation and instability, whereas administration of exogenous IL-10 or gene transfer strategies can substantially reduce lesion size and improve plaque stability on the animal models of atherosclerosis (62). Where as, Zhang L.et al in their study on humans have demonstrated that long-term prognosis that are favourable with a decreased incidence of death and nonfatal myocardial infarction in ACS patients with mild to moderate coronary artery lesions (64) are linked to higher IL-10 levels.

3.1.3.3c Other Important Inflammatory Markers in CAD Research

The inflammatory cascade in the pathogenesis is complex and also involves other markers such as Oxidized Low-Density Lipoprotein (oxLDL) and Lectin-like oxLDL Receptor-1 (LOX-1) which may be independently associated with increased CAD risk, plaque vulnerability, and adverse cardiovascular events (65).

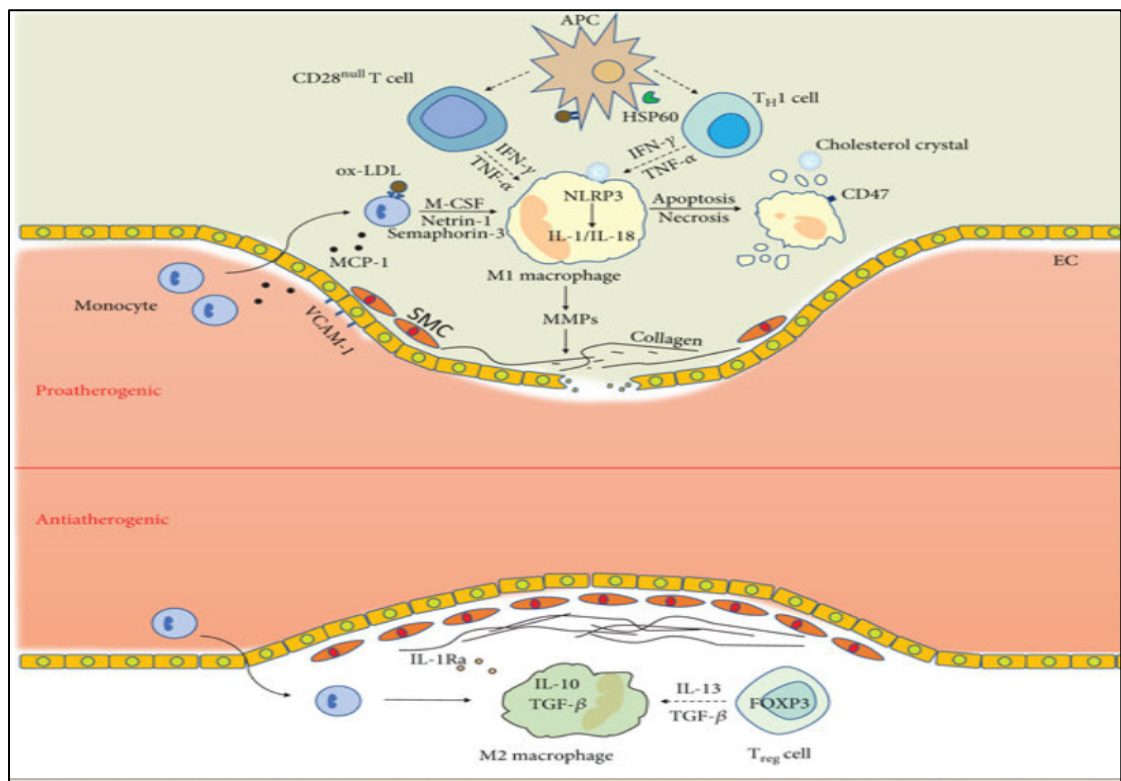
Another marker of inflammation, Soluble CD40 Ligand (sCD40L) plays a crucial role as also Pentraxin 3 (PTX3), an acute-phase protein produced by various cells, including endothelial cells, macrophages, and smooth muscle cells. A member of long pentraxin family, PTX3 acts directly at sites of inflammation. Both the markers are known to initiate and propagate inflammatory and thrombotic responses within the atherosclerotic plaque and raised levels are correlated with higher risk of ACS and MACE, particularly in individuals with unstable angina (66-67).

Many recent research have emerged which have recognized markers which are all known to be associated with atherosclerotic plaque burden, plaque instability, and adverse cardiovascular outcomes in CAD patients. Some of the important markers are

- Fibrinogen (68)
- Growth Differentiation Factor 15 (GDF-15) which is a cytokine responsive to stress (69).
- sICAM-1, sVCAM-1 which are the soluble forms of cell adhesion molecules, such as and also E-selectin (70).

- by a complex interplay between pro-inflammatory and anti-inflammatory cytokines. A shift towards a pro-inflammatory profile contributes to endothelial dysfunction, plaque instability, and adverse cardiac events among the ACS (Acute Coronary Syndrome) patients (73).

Figure 3.5: Immune and inflammation pathways in the pathogenesis of ACS (73)



3.2. Diet and CAD

3.2.1: Dietary patterns and CAD

Coronary artery disease continues to exhibit a global increase, notably among Asian Indians, with adverse prognosis linked to the progression of coronary lesions (74).

The complex etiology of CAD underscores numerous modifiable risk indicators, many of which are amenable to effective interventions (75).

Lifestyle factors, including smoking, body mass index, and systolic blood pressure, collectively account for a significant portion of population-level changes in coronary heart disease (CHD) rates and mortality(76).

Consequently, essential lifestyle modifications such as refraining from tobacco, engaging in physical activity, and adopting a balanced and healthy eating pattern are crucial for diminishing cardiovascular disease (CVD) risk (77).

3.2.2: Diet as a Major Determinant of CAD Development and Progression

Numerous studies, encompassing epidemiological investigations, randomized clinical trials (RCTs), cross-cultural, cohort, and intervention studies, consistently highlight diet as a major determinant of CHD occurrence, progression, and mortality (78,79).

Substantial reductions in CHD risk can be achieved through dietary and lifestyle modifications in both high-risk individuals and cardiac patients (76).

The past decades have seen an evolution in nutritional research with reference to the CVD prevention and has documented a shift towards research based on dietary patterns rather than a single nutrients and/or specific foods, where the focus was often on isolating the effects of individual nutrients (e.g., saturated fat, cholesterol) or specific food items (e.g., eggs, red meat) on health outcomes. While foundational, this approach often overlooked the complex interactions between various dietary components and that people consume combinations of foods, and the synergistic or antagonistic effects of these combinations (i.e., dietary patterns) are more relevant to overall health than individual components. However the improvements in the cardiometabolic health and the optimal components of a diet to achieve it remain uncertain (80).

Moreover , assessing overall dietary patterns offers more informative insights into the correlation between total dietary intake and disease risk compared to scrutinizing individual food components(81,82).These patterns effectively reflect actual dietary behaviour, providing comprehensive findings on diet-health relationships.

3.2.3: Dietary Patterns and CAD Risk: Unhealthy vs. Healthy Patterns

Research consistently links specific unhealthy dietary patterns to increased CAD risk and severity. For instance, the Quasi-Western and Sugar-Fast foods patterns are significantly and positively correlated with CAD risk in both males and females (83).

The Western dietary pattern is globally associated with an elevated risk of CVDs (84). This pattern, also marked by increased consumption of refined carbohydrates, fat, animal based products, and limited intake of plant based food items, positively correlates with severity of CAD (85-86).

In the Indian context, specific dietary changes have been implicated in CAD trends (87).

Patients with triple vessel disease (TVD) often exhibit a higher intake of carbohydrates and fat (specifically palmitic acid), alongside lower protein and fiber intake, compared to those with single or double vessel disease (88). High carbohydrate consumption and high-fat diets have been linked to weight gain and obesity, established CAD risk factors (89).

Conversely, a low-fat diet is associated with slower CAD progression (90) and monounsaturated fatty acids (MUFA) are recognized as protective against CAD progression (91). While some protein studies show conflicting results depending on protein source (92) plant-based diets demonstrate potential in decelerating TVD progression (93).

3.2.4: Impact of Diet on Systemic Inflammation and CAD

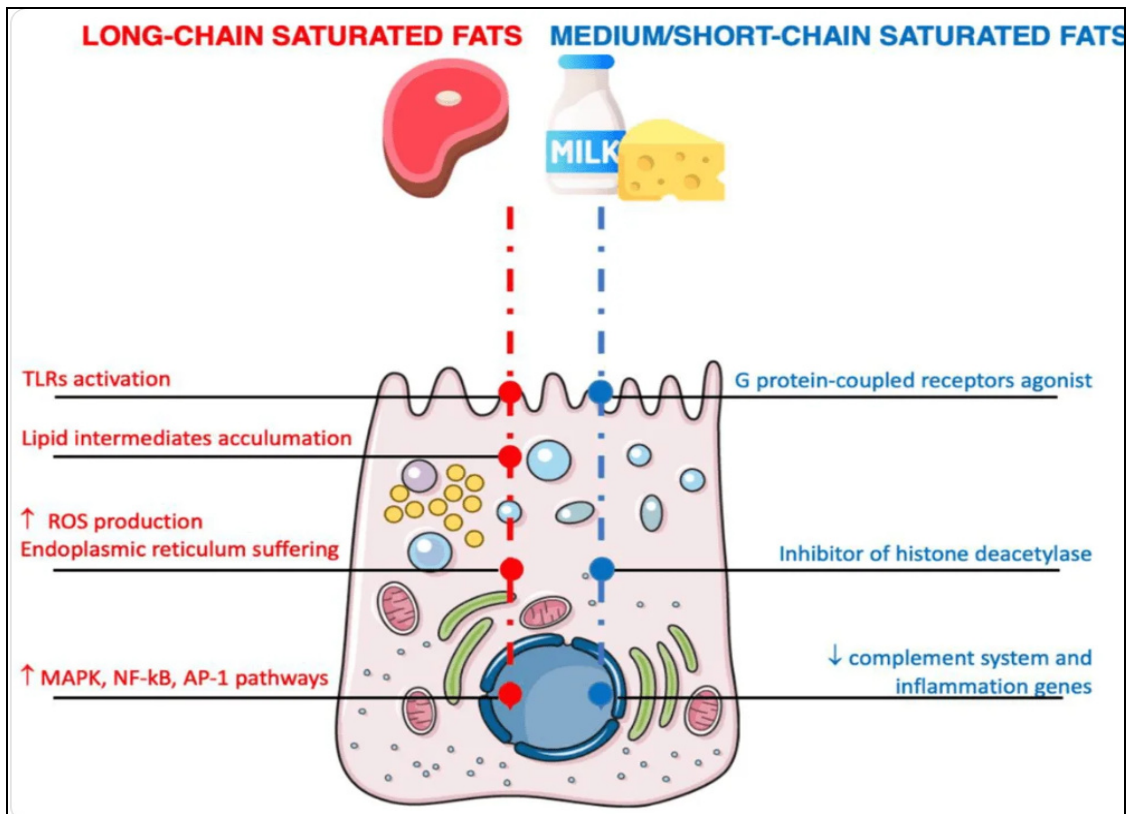
The underlying pathogenesis of CAD is a chronic low-grade inflammation. Our dietary choices play a pivotal role in either promoting or ameliorating this inflammatory state.

3.2.4a: Pro-inflammatory dietary components and Chronic Low-Grade Inflammation.

Specific dietary components are well-established instigators of chronic low-grade inflammation, contributing to a pro-inflammatory milieu within the body. The mechanism of action of some of them are detailed below

- i) **Saturated Fats:** Diets rich in saturated fatty acids (SFAs) which are commonly found in red meat, full-fat dairy products, are potent activators of inflammatory pathways. SFAs can directly interact with the immune cells through their action on Toll-like receptor 4 (TLR4) and adipocytes and triggers downstream signaling cascades, including the activation of nuclear factor-kappa B (NF- β). This leads to the transcription and production of numerous pro-inflammatory cytokines such as TNF- α and IL-6, both of which are elevated in CAD patients and positively correlate with hsCRP levels (94).

Figure 3.6: Effect of saturated fatty acids on intestinal cells and inflammatory pathways.(94)



Furthermore, Zou, J., et.al, observed that the SFAs contribute to endoplasmic reticulum (ER) stress and mitochondrial dysfunction which then activate the NLRP3 inflammasome. Activated NLRP3 inflammasome synthesises mature IL-1 β and IL-18, potent pro-inflammatory cytokines (95).

- i) Refined Carbohydrates and Sugars:** High intake of processed carbohydrates and added sugars (e.g., sucrose, high-fructose corn syrup) rapidly increase blood glucose and insulin levels. Chronic hyperglycemia causes activation of various metabolic pathways, including the polyol pathway and protein kinase C which leads to increased production of reactive oxygen species (ROS) and oxidative stress. Oxidative stress, in turn, activates NF- κ B and other pro-inflammatory signaling pathways which has several pro-inflammatory effects. The non-enzymatic reaction of sugars with proteins or lipids form the advanced glycation end products (AGEs) which accumulate in tissues and bind to their receptor (RAGE), triggering further oxidative stress and inflammatory responses, contributing to vascular dysfunction and mortality in patients of CAD (96-97). Excessive sugar intake can also contribute to *de novo*

lipogenesis in the liver, leading to increased hepatic SFA synthesis (dyslipidemia) and subsequent inflammatory signaling (98).

- ii) Processed Foods:** Processed foods often combine high levels of SFAs, refined carbohydrates, added sugars, and sodium, along with various food additives and emulsifiers. These components synergistically promote inflammation.

Mozaffarian, D. et al, noted that trans fatty acids are potent inflammatory agents that elevate systemic inflammation and are increasingly used in processed foods that has partially hydrogenated oils (99). Also many processed foods contain emulsifiers and artificial sweeteners that can disrupt gut microbiota composition, leading to gut dysbiosis.

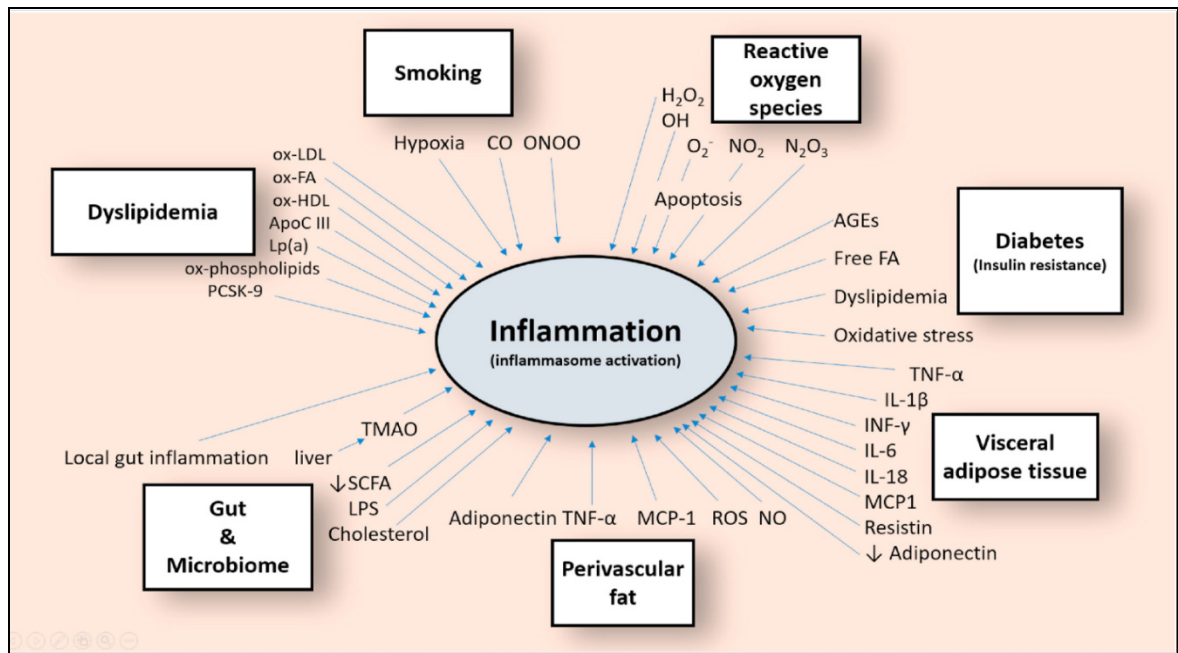
Mechanisms of Proinflammatory Component-Driven Chronic Inflammation:

- a) Oxidative Stress:** As mentioned, pro-inflammatory diets, rich in SFAs, refined carbohydrates, and processed foods, can significantly increase the generation of reactive oxygen species (ROS). This imbalance between ROS production and antioxidant defenses leads to oxidative stress, which acts as a powerful signaling molecule, activating numerous pro-inflammatory pathways, including NF- κ B and AP-1, and contributing to endothelial dysfunction in CAD (96).
- b) Gut Dysbiosis:** The composition of the gut microbiota is profoundly influenced by diet. Diets high in processed foods, refined carbohydrates, and SFAs tend to reduce the diversity of beneficial gut bacteria and promote the growth of pro-inflammatory species. This gut dysbiosis can lead to increased gut permeability ("leaky gut"), allowing bacterial products like LPS to translocate into the systemic circulation. Circulating LPS then binds to TLR4 on immune cells, initiating a robust inflammatory response characterized by increased production of TNF- α , IL-6, and other pro-inflammatory cytokines, directly impacting systemic inflammation and contributing to CAD progression (100).
- c) Adipose Tissue Inflammation:** Obesity, often exacerbated by pro-inflammatory diets, is characterized by chronic low-grade inflammation within adipose tissue. Adipocytes, particularly when hypertrophied, release pro-inflammatory adipokines (e.g., TNF- α , resistin) and attract macrophages, which infiltrate the adipose tissue and differentiate into pro-inflammatory M1 macrophages. These M1 macrophages release further inflammatory cytokines, creating a self-perpetuating cycle of local and

systemic inflammation, impacting insulin sensitivity and contributing to the overall inflammatory burden in CAD patients (101).

- d) Activation of Inflammatory Pathways: NF- β :** This transcription factor is a central mediator of inflammatory responses. SFAs, AGEs, and bacterial LPS all activate NF- β , leading to the upregulation of genes encoding pro-inflammatory cytokines, chemokines, and adhesion molecules, all contributing to systemic inflammation and atherosclerosis (94,97).
- e) Inflammasomes:** NLRP3 inflammasome is a multi-protein complex that, upon activation, can cause the cleavage and secretion of potent pro-inflammatory cytokines like IL-1 β and IL-18. It is activated by dietary components like SFAs, high glucose levels, and cholesterol crystals and other conditions like dyslipidemia, DM, obesity thereby driving sterile inflammation crucial in the development and progression of CAD (47,95).

Figure 3.7: Activation of Inflammasomes (47)



3.2.4b: Anti-inflammatory Dietary Components Modulate chronic low grade Inflammation

Conversely, specific dietary components possess potent anti-inflammatory properties, capable of modulating and dampening these pro-inflammatory pathways.

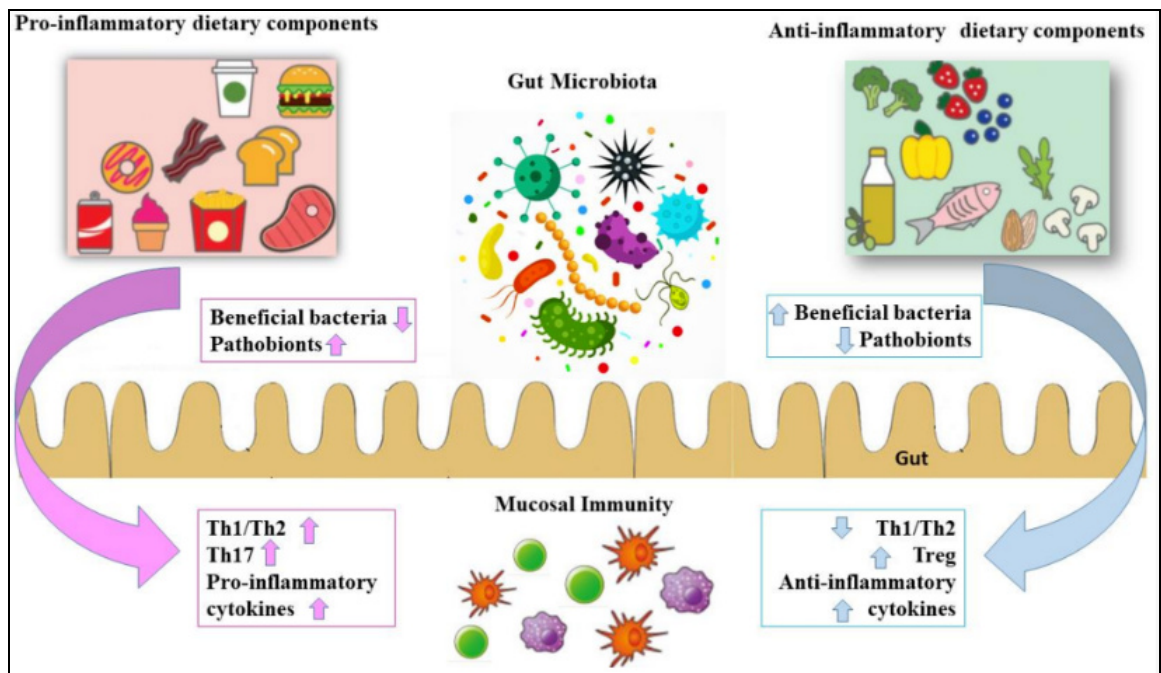
- i) **Omega-3 Fatty Acids:** Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and certain algal oils, are well-known for their anti-inflammatory effects. EPA and DHA are primarily found in fatty fish like salmon, mackerel. They compete with arachidonic acid (an omega-6 fatty acid) for metabolism by Cyclooxygenase and lipoxygenase enzymes, leading to the production of less inflammatory eicosanoids. More importantly, omega-3 fatty acids are precursors to specialized pro-resolving mediators (SPMs) such as resolvins, protectins, and maresins. These SPMs actively promote the resolution of inflammation by inhibiting neutrophil infiltration, enhancing efferocytosis (clearance of apoptotic cells), and reducing pro-inflammatory cytokine production. Omega-3 fatty acids can also directly suppress NF- κ B activation and reduce the expression of adhesion molecules, thereby mitigating systemic inflammation and improving endothelial function, leading to a reduction in hsCRP and TNF- α levels, and an increase in IL-10 (an anti-inflammatory cytokine) (102).

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- ii) Fiber (Soluble and Insoluble):** High-fiber diets are associated with a reduced incidence of metabolic disorders, including heart disease (103). Dietary fiber, abundant in whole grains, legumes, fruits, and vegetables, exerts its anti-inflammatory effects primarily through its impact on the gut microbiota. The beneficial gut bacteria use the soluble fibers as a fermentable substrate which helps in the production of short-chain fatty acids (SCFAs), particularly butyrate, propionate, and acetate. Butyrate, in particular, has potent anti-inflammatory properties, including the ability to inhibit histone deacetylases (HDACs), which can lead to suppression of NF- β and reduced production of pro-inflammatory cytokines. SCFAs also strengthen the intestinal barrier, reducing gut permeability and thus limiting the translocation of LPS and other inflammatory molecules into the systemic circulation. This contributes to a healthier gut microbiome profile, potentially increasing beneficial bacteria that produce SCFAs and reducing pro-inflammatory species, leading to a decrease in systemic inflammation markers (104).
- iii) Polyphenols from Fruits and Vegetables:** Fruits and vegetables are rich sources of polyphenols, a diverse group of plant compounds with significant antioxidant and anti-inflammatory properties. Examples include flavonoids - quercetin, anthocyanins, phenolic acids, and resveratrol.

Mechanisms of Anti-inflammatory dietary Component-Driven Chronic Inflammation:

- a) Antioxidant Activity:** Polyphenols directly scavenge free radicals, reducing oxidative stress and subsequently inhibiting the activation of redox-sensitive inflammatory pathways like NF- β (105).
- b) Direct Modulation of Inflammatory Pathways:** The activity of pro-inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX) can be inhibited by many polyphenols which reduces the production of prostaglandins and leukotrienes. They can also suppress NF- β activation and interfere with inflammasome assembly, thereby reducing the production of pro-inflammatory cytokines. (106).

Figure 3.8: Interaction of diet and gut microbiome on systemic inflammation.(107)



Gut Microbiota Modulation: The nutrient as well as the non-nutritive bioactive elements in the diet have a significant effect on the processes which can cause immune modulation and inflammation. The gut microbiome can also cause biotransformation of food elements into active metabolites. These metabolites along with the food components can modulate ongoing metabolic responses or the entire the immune system now known as the “microbiome–inflammation or microbiota–immunity axis”. They can also selectively promote the growth of beneficial gut bacteria, contributing to a healthy gut ecosystem and reduced systemic inflammation (107).

In conclusion, dietary choices profoundly impact systemic inflammatory pathways directly impacting key biomarkers like hsCRP, TNF-alpha, and IL-10. Pro-inflammatory diets exacerbate inflammation, leading to elevated hsCRP and TNF-alpha and suppressed IL-10, which mechanistically contribute to endothelial dysfunction, a prothrombotic state, and plaque instability, thereby increasing the risk of MACEs and mortality in CAD patients. Conversely, anti-inflammatory dietary patterns effectively reduce these pro-inflammatory markers and enhance anti-

inflammatory responses, leading to a significant reduction in CAD risk and improved clinical outcomes. For patients with CAD, understanding and implementing these dietary principles is paramount in managing systemic inflammation ultimately contributing to reduced MACEs and better cardiovascular outcomes.

3.3 Dietary Inflammatory Index (DII)

3.3.1 Evolution of Dietary Research in Nutritional Epidemiology

“The majority of dietary indexes in epidemiological research prior to the development of the Dietary Inflammatory Index (DII), except the glycaemic index (108) typically fell into three main categories:

- 1) Those grounded in dietary guidelines such as the Healthy Eating Index–2010 or the Alternative Healthy Eating Index, both derived from the US Dietary Guidelines (109), or the Dietary Approaches to Stop Hypertension (DASH) (110) recommended by the National Heart, Lung, and Blood Institute;
- 2) Those linked to adherence to a specific foodway or cuisine, like the Mediterranean Dietary adherence score (111). Or
- 3) Those developed from a particular study using regression techniques such as principal components analysis or reduced rank regression (112).

These methods are easy to create, but they often don't capture the full range of differences in exposure—a frequent challenge in nutritional epidemiology (113). Furthermore, Del Razo Olvera FM et.al, noted that method-specific problems arise because dietary guidelines are not always based on the strongest empirical evidence and are being subject to debate, controversy, and occasional revision (114).

Gupta R et.al, observed that although Mediterranean dietary recommendations may have health benefits, the Mediterranean region comprises 21 countries with diverse cuisines, introducing complexity to its interpretation. Additionally, several healthy diets globally do differ from the Mediterranean pattern, such as South Asian (115) or East Asian diets(116).

Moreover, the repeated application of specific study- and population-derived indexes in the same or similar population (117) poses a risk of inflated measures of association due to intra-method correlated errors and might not be easily generalizable across different populations, a concern previously noted by researchers (118). This can

lead to inaccuracies in attributing a significant portion of the variance to the index score.

In contrast, the DII was meticulously designed to incorporate evidence from diverse human populations, employing varied study designs and dietary assessment methods. Beyond human studies, the DII integrates evidence from qualifying laboratory animal and cell culture experiments, albeit with lower weighting (119). The rapid progress in understanding inflammation's role in health (120) and the impact of diet on inflammation (121) set the stage for creating the Dietary Inflammatory Index (DII). The DII is a continuous score developed by Shivappa N and colleagues. It is unique in that it's specifically designed to quantify the inflammatory potential of an individual's diet based on a comprehensive literature review of 45 food parameters (nutrients, food components, and whole foods) and their known associations with six inflammatory biomarkers - IL-1 β , IL-4, IL-6, IL-10, TNF- α , and C-reactive protein (CRP).

A higher DII score indicates a more pro-inflammatory diet, while a lower (more negative) score indicates an anti-inflammatory diet. This is arguably the most direct index linking diet to inflammation and subsequently to NCDs. Numerous studies have shown that higher (more pro-inflammatory) DII scores are associated with an increased risk of various NCDs, including cardiovascular diseases, metabolic syndrome, diabetes, certain cancers, and cognitive disorders, primarily by reflecting a diet that promotes chronic inflammation(122).

3.3.2 Definition and Calculation of DII

The Dietary Inflammatory Index (DII) serves as a robust, literature-derived tool designed to comprehensively quantify the net inflammatory potential of an individual's diet. It is uniquely established by integrating a vast body of evidence on the inflammatory and anti-inflammatory effects of various dietary components, moving beyond single nutrients to assess the holistic dietary impact on key inflammatory biomarkers, and has been widely validated across diverse populations and numerous health outcomes (122).

In 2014, “Shivappa et al. introduced the dietary inflammatory index (DII), incorporating information from literature on 45 food parameters and their correlation with six inflammatory blood markers: interleukin (IL)-1 β , IL-4, IL-6, IL-10, tumor-necrosis-factor alpha (TNF)- α , and C-reactive protein” (Shivappa et al., 2014). The computation of the literature-derived, population-based Dietary Inflammatory Index

(DII) by Shivappa et al has been extensively explained Shivappa et al., 2014 (123). “To propose application and the DII calculation, the fundamental assumptions and steps of DII calculation are succinctly outlined. First, fixed relationships are posited between the considered inflammatory markers (pro-inflammatory: IL-1 β , IL-6, TNF- α , C-reactive protein; anti-inflammatory: IL-4, IL-10) and the DII. Second, articles providing results on the influence of food parameters on these inflammatory markers are chosen for effect score calculation. Third, global values (means and standard deviations) for the considered food parameters are derived from daily consumption data of a global database based on 11 different countries. Additionally, P is assumed as the set of food parameters” (a total of 45 food parameters were used) included in the DII calculation (123)

“For the scoring of selected articles with information on the influence of the 45 food parameters on the six pro-/anti-inflammatory blood markers, let x be the result of an article and $a: X \rightarrow \{-1, 0, 1\}$ be defined through”

$$a(x) = \begin{cases} -1, & x = \text{food parameter showed anti-inflammatory effect} \\ 0, & x = \text{food parameter showed no inflammatory effect} \\ 1, & x = \text{food parameter showed pro-inflammatory effect} \end{cases}$$

“For a specific food parameter (p) , the scores $(a(x_{pi}))$ from the chosen articles $(x_{pi}, i = 1, \dots, n)$ for effect score calculation are weighted using weights $(w(x_{pi}))$, contingent on study characteristics (study type, study design), such as a study being human and having a prospective cohort design. This process leads to the computation of the raw inflammatory effect score (RIES) as follows” :

$$RIES_p = \frac{\sum_{i=1}^n a(x_{pi}) \times w(x_{pi})}{\sum_{i=1}^n w(x_{pi})} \in [-1, 1].$$

The adjusted RIES, the overall inflammatory effect score (OIES), is calculated by

i) if $\sum_{i=1}^n w(x_{pi}) < \text{median}((\sum_{i=1}^n w(x_{pi}))_{p \in P})$:

$$\begin{aligned} OIES_p &= \frac{\sum_{i=1}^n w(x_{pi})}{\text{median}((\sum_{i=1}^n w(x_{pi}))_{p \in P})} \times RIES_p \\ &= \frac{\sum_{i=1}^n w(x_{pi})}{\text{median}((\sum_{i=1}^n w(x_{pi}))_{p \in P})} \times \frac{\sum_{i=1}^n a(x_{pi}) \times w(x_{pi})}{\sum_{i=1}^n w(x_{pi})} \\ &= \frac{\sum_{i=1}^n a(x_{pi}) \times w(x_{pi})}{\text{median}((\sum_{i=1}^n w(x_{pi}))_{p \in P})} \in (-1, 1) \end{aligned}$$

ii) if $\sum_{i=1}^n w(x_{pi}) \geq \text{median}$

$$OIES_p = RIES_p.$$

The calculation for the global daily consumption reference for every food parameter p is

$$\bar{I}_p = \frac{1}{I} \sum_{i=1}^I I_{p,i},$$

where $(I_{p,i})$ represents the quantity of the daily consumption of the specified food parameter for the subject i from a global database. “Standard deviation determines the corresponding global variability and is noted as the amount as in the same unit of consumption of the particular food parameter for i subject from the generated global database”.

Global variability is given by the standard deviation

$$sd_p = \sqrt{\frac{1}{I-1} \sum_{i=1}^I (I_{p,i} - \bar{I}_p)^2}.$$

The reported amount of the daily consumption $I_{p,i}$ of a particular food parameter p and subject i for which the DII should be calculated is standardized by

$$Z_{p,i} = \frac{I_{p,i} - \bar{I}_p}{sd_p}.$$

The DII for a particular food parameter p and subject i results from

$$DII_{p,i} = (2 \times \Phi(Z_{p,i}) - 1) \times OIES_p \in [-1, 1],$$

Where Φ is the standard normal distribution function.

Finally, the DII for the i -th subject is then calculated by

$$DII_i = \sum_{p \in P} DII_{p,i} \in [-|P|, |P|],$$

Completing the original calculation method by Shivappa et al. 2014 (123).

The DII has been widely utilized in over 200 human studies, encompassing diverse ethnicities and health outcomes (124). Vitale et al., noted that “Numerous investigations have identified associations between a pro-inflammatory diet (positive DII score) and an elevated risk of various non-communicable diseases (NCDs), such as certain cancer types, cardiovascular disease, or type 2 diabetes” (125). As a potentially cost-effective and user-friendly tool, the DII holds promise for global application to evaluate dietary habits and mitigate NCD development by promoting a more non-/anti-inflammatory eating behaviour.

3.3.3 Importance of DII in Assessing Dietary Inflammation

The Dietary Inflammatory Index (DII) is an effective tool to quantify an individual's dietary inflammation, where a more pro-inflammatory dietary intake is indicated with higher scores indicating a and otherwise. Its widespread application has highlighted the critical links between dietary choices and adverse health outcomes across diverse populations.

DII, systemic inflammation, CAD severity and CVD related Mortality

Globally, a consistent body of evidence demonstrates that a strong association exists between a pro-inflammatory diet and elevated risk of all-cause mortality and CVD-related mortality. Meta-analyses of various prospective cohort studies from different continents have shown that individuals in the highest DII tertiles or quintiles face a significantly elevated risk of all cause mortality, as well as specific cardiovascular mortality (126-127). For instance, a meta-analysis encompassing numerous studies reported a positive association between higher DII scores and an elevated risk of CVD incidence and mortality (126). This global trend underscores that irrespective of geographical location, a diet rich in pro-inflammatory components contributes to a systemic inflammatory state that accelerates disease progression and heightens mortality risk. Beyond mortality, pro-inflammatory diets have a demonstrable impact on the severity of CAD. Studies utilizing angiographic scoring systems, such as the Gensini score, reveal a direct relationship between a higher DII and more severe coronary artery lesions (128).

Additionally, elevated DII scores have been consistently associated with an increased incidence of MACEs and has a crucial clinical consideration (127). The chronic inflammatory milieu induced by such diets destabilizes atherosclerotic plaques,

making them more prone to rupture. The global narrative of DII and CVD outcomes takes on particular urgency when examining the Indian and broader Asian populations. These populations exhibit a disproportionately high and often premature burden of CAD, even when traditional risk factors are accounted for (129). This "Asian Indian paradox" or the higher susceptibility to CAD at a younger age, often presenting with diffuse and severe disease, points towards additional contributing factors, among which dietary patterns and their inflammatory potential are increasingly recognized (130). The concept of "thin-fat" phenotype prevalent in South Asians, characterized by higher body fat despite lower BMI, is also associated with increased systemic inflammation and insulin resistance, further exacerbated by pro-inflammatory diets (131). Across Asia, varying dietary transitions and genetic predispositions interact with the inflammatory potential of diets. While some traditional Asian diets, such as those emphasizing fish, vegetables, and whole grains, are inherently anti-inflammatory, rapid Westernization of dietary habits is a growing concern. Studies from East Asia and Southeast Asia are increasingly demonstrating similar associations between higher DII scores and increased risk of CAD, stroke, and overall mortality (132-133).

For example, research from Iran has directly linked higher DII scores to a greater likelihood of severe CAD, as assessed by the Gensini score, reinforcing the impact of inflammatory dietary patterns on disease pathology in an Asian context (127). These findings underscore that despite regional variations in dietary components, the overall inflammatory potential of the diet remains a crucial determinant of cardiovascular health outcomes across the Asian continent.

Traditional Indian diets, particularly in urbanizing areas, have undergone significant transitions, often incorporating higher amounts of refined carbohydrates, unhealthy fats (e.g., from processed snacks, excessive use of adulterated ghee in certain contexts), and less diverse plant-based foods, despite a historical emphasis on vegetarianism. This shift contributes to a pro-inflammatory dietary profile. Studies in Indian populations, while perhaps less numerous than in Western cohorts specifically utilizing the DII, strongly indicate that dietary habits are linked to elevated hsCRP and increased prevalence of metabolic syndrome, a key driver of inflammation and CAD (134-135). Our research is specifically focused on quantifying DII in CAD patients and correlating it with hsCRP, IL-10, and TNF-alpha to provide localized, granular evidence for this critical link.

3.4 North Karnataka Food Pattern and CAD

Coronary Artery Disease is a growing concern in India, often striking people prematurely. Understanding regional dietary influences is crucial, and North Karnataka, with its distinct food culture, represents a key, yet under-researched, area for its impact on CAD risk, progression, severity, and mortality.

3.4.1 North Karnataka Food: Traditional and Evolving Food Components

North Karnataka's traditional cuisine is largely dependent on jowar (sorghum), bajra (pearl millet), and wheat as staple grains for flatbreads. Legumes (dals, pulses) provide significant protein and fiber. Seasonal, local vegetables are common, and groundnut (peanut) oil, rich in monounsaturated fatty acids (MUFAs), is a primary cooking medium, though usage and frying methods vary.

Milk products (curds, buttermilk) and spices are also widely consumed, offering flavor and potential bioactive compounds.

However, modern dietary shifts are clear. Refined flours, processed foods, sugary beverages, and oils high in saturated or trans fats are increasingly prevalent, especially in urban and semi-urban areas. The use of hydrogenated vegetable fats (vanaspati) for deep-frying and sweets in homes and eateries marks a notable departure from traditional practices.

3.4.2 Regional Dietary Habits and Preferences

Traditional North Karnataka diets are largely vegetarian or ovo-vegetarian, emphasizing whole grains, legumes, and fresh produce. Meals typically feature jowar or wheat rotis with curries (palya) and dals. Jaggery is a common traditional sweetener, though refined sugar intake is on the rise. A significant regional preference is the preparation of various deep-fried snacks and sweets, particularly during festivals. These often incorporate refined flour, sugar, and unhealthy fats. The shift from traditional stone-ground flours to industrially processed ones, and from whole pulses to refined dals, also impacts the diet's nutritional and inflammatory potential. The widespread consumption of mirchi bhaji (chilli fritters) and other fried snacks, often prepared with reused oil, carries substantial implications for cardiovascular health.

3.4.3 North Karnataka Food Patterns and CAD Outcomes

While comprehensive epidemiological studies directly linking the Dietary Inflammatory Index (DII) to CAD outcomes in North Karnataka are limited, existing knowledge about regional dietary components, combined with broader evidence, allows us to hypothesize their inflammatory impact on heart health. The traditional emphasis on whole grains and legumes offers anti-inflammatory benefits as they tend to promote the growth of good gut microbiome due to their high fiber content. SCFAs which are produced by the gut bacteria have systemic anti-inflammatory effects and bolster gut barrier integrity (104-136). Conversely, the rising consumption of refined carbohydrates, trans fats, and saturated fats represents a significant shift towards a pro-inflammatory dietary profile (137-138). Even with groundnut oil's favorable MUFA profile, its oxidative stability and reuse practices warrant attention, as these can generate harmful inflammatory compounds (139). Moreover, frequent intake of deep-fried items, especially those containing trans fatty acids, is known to worsen systemic inflammation and dyslipidemia, directly impacting CAD risk and progression (99). The inflammatory pathways are further exaggerated by the formation of advanced glycation end-product (AGE) and sugars, whether jaggery or refined table sugar contribute to their formation and lead to oxidative stress (97).

These dietary patterns likely elevate inflammatory markers like hsCRP and TNF-alpha, while potentially suppressing anti-inflammatory IL-10, thereby accelerating atherosclerosis and increasing CAD severity in CAD patients. This sustained inflammation destabilizes atherosclerotic plaques, raising the risk of MACEs like heart attacks and strokes, and ultimately increasing cardiovascular mortality [97].

The present sincere and focused research effort was critically carried out to:

- Precisely quantify the inflammatory potential of dietary practices (DII) among North Karnataka (both traditional and evolving) CAD cohort.
- Reverse identify specific local dietary components that either drive or mitigate inflammation. Which can in future help develop a culturally appropriate, region-specific dietary guidelines to effectively reduce CAD risk, morbidity and mortality and inform targeted public health campaigns to combat the rising tide of CAD in North Karnataka. Without this dedicated investigation, the unique dietary landscape of North Karnataka—balancing traditional protective elements with emerging

inflammatory influences—remains a critical gap in our understanding of CAD epidemiology, prevention and prognosis in the region.

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CHAPTER 4

MATERIALS AND

METHODS

4.1 Study design:

This was a hospital based observational, prospective cohort study.

4.2 Study Period:

The study was conducted from JULY -2020 to DEC 2022 for a period of 2 years.

4.3 Source of data:

The study was conducted in the department of Biochemistry and General Medicine/Cardiology, at BLDE (DU), SBMP Medical College, Vijayapura. Karnataka. The patients who attended the Cardiology OPD and IPD were selected for the study.

4.4 - Sample size:

A total 310 CAD patients were enrolled for the study

4.5 Sample size calculation:

The calculation for sample size was done according to the Cox proportional hazard model (reference) using the formula

$$n_{event} \geq \frac{2 \left(Z_{1-\alpha/2} + Z_{1-\beta} \right)^2}{(\log_e HR)^2}$$

$$\alpha=0.05$$

$$\beta=0.2$$

HR= Hazard Ratio =1.27, the minimum number of events n_{event} needed was approximately 276. We added 10% for loss at follow up, the minimum sample size was 306.

As the incidence of MACE s has no published data in the region under study, the data was obtained by the experience of the consultants in medicine and cardiology which was below 5% at a follow-up of 1 week and 2% at 30 days follow-up(1).

4.6 Ethical clearance:

The study received approval from the Institutional Ethics Committee of BLDE (Deemed to Be University), Vijayapura, under reference number IEC-BLDE (DU) IEC/413/2019-20. The written informed consent was obtained by all the participants prior to their enrollment, following a detailed explanation of the study procedures. All the principles outline in the Declaration of Helsinki were followed for the research protocol.

4.7 Inclusion criteria and Exclusion criteria

4.7.1 Inclusion criteria:

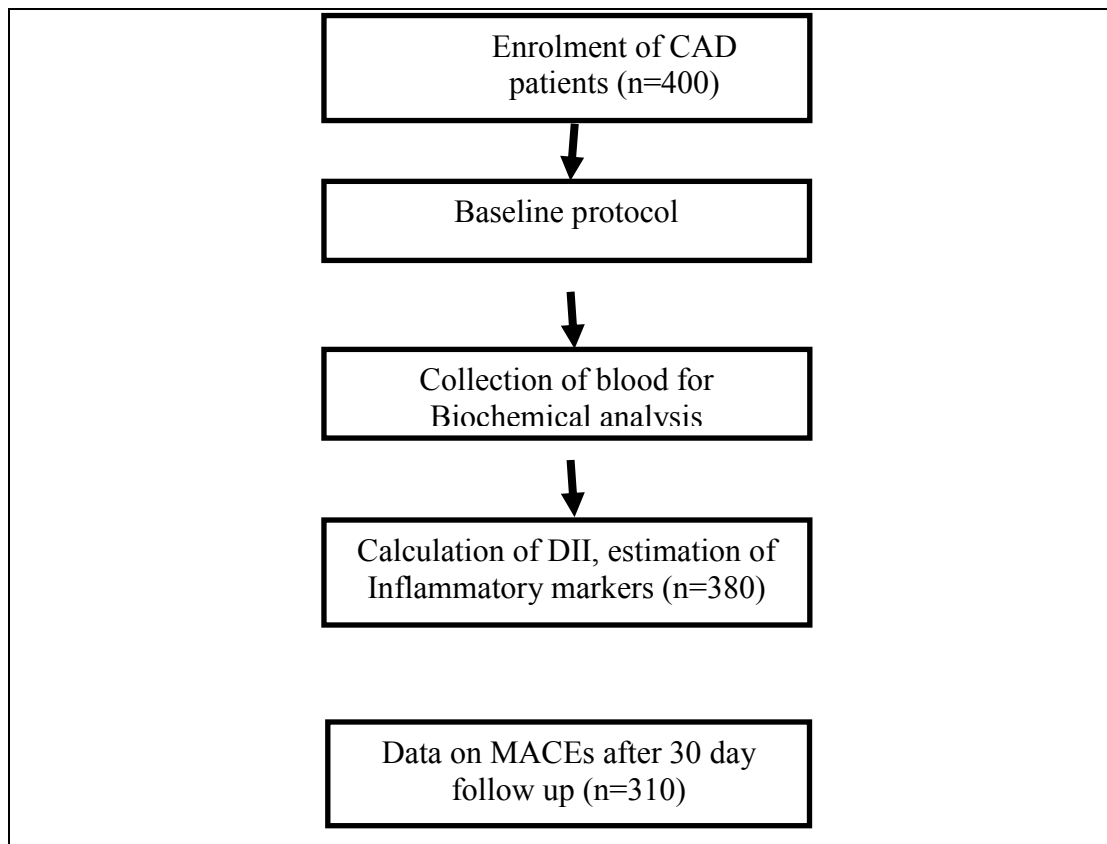
Subjects (18-76 yr) presenting with angina at the outpatient departments of a tertiary care Hospital, and Research Center under the care of the consultant physician or cardiologist and diagnosed as definite CAD were enrolled in the study. Both the asymptomatic and CAD patients with complications like acute coronary syndrome (unstable angina or MI), congestive heart failure and cardiac arrhythmias were included.

4.7.2 Exclusion criteria:

Subjects with a history of cerebrovascular disease, or systemic disorders, such as severe hepatic, renal diseases and any condition that affects the inflammatory status were excluded from the study. Further, pregnant women and the participants with chronic infection ($WBC > 10,000/mm^3$) and chronic renal failure (GFR 90 ml/min per 1.73 m² or less) were also excluded.

4.8 Study protocol:

Figure 4.1: Summary of study protocol



4.8.1 Definitions that were applied.

a. CAD: CAD was defined as those who satisfied the diagnosis of definite CAD and will be based on any of: “documented evidence of prior acute coronary syndrome (ACS) or treatment for CAD, documented history of coronary angioplasty or CABG, more than 50 % epicardial coronary stenosis by invasive coronary angiography, ECG showing pathological Q waves (any of Minnesota code 1-1-1 to 1-1-7 or 1-2-1 to 1-2-5 or 1-2-7), imaging evidence of a region of loss of viable myocardium that is thinned and has a motion abnormality, in the absence of a non-ischemic cause” as stated by Mendis S et.al, 2011 (2).

b. Angina pectoris: The angina is classified according to Diamond classification as typical angina or atypical angina if the chest pain characteristics meet all 3 or 2 of the below criteria, respectively and is defined as “substernal discomfort, heaviness, or a pressure-like feeling, which may radiate to the jaw, shoulder, back, or arm and which

typically lasts several minutes. These symptoms are usually brought on by exertion, emotional stress, cold, or a heavy meal and are relieved by rest or nitroglycerin within minutes” as given by (Diamond GA 1983) (3).

c. Major adverse cardiovascular event (MACEs) : Young, J.B., et al,2018 help identify MACEs as “Events which comprised non-fatal MI, non-fatal stroke, and CVD-related death (death occurring within 30 days after a diagnosis for MI, stroke, unstable angina, heart failure, sudden cardiac arrest, cardiogenic shock, other cerebrovascular or cardiovascular events)” (4).

d. Diabetes Mellitus: Defined as “fasting blood glucose value of ≥ 7 mmol/L and/or if there was current use of medications for diabetes” by American Diabetes Association (5).

e. Hypertension: Defined as “blood pressure ≥ 140 mm of Hg systolic and/or ≥ 90 mm of Hg diastolic and/or currently on drugs for high blood pressure” by JNC 7 Report 2003. (6), and

f. Dyslipidemia: Defined “as any of: serum total cholesterol ≥ 5.18 mmol/L, serum LDL cholesterol ≥ 3.37 mmol/L, serum HDL cholesterol < 1.04 mmol/L in men or < 1.29 mmol/L in women, or serum triglycerides ≥ 1.69 mmol /L” given by NCEP and Adult Treatment Panel III (7).

g. Abdominal obesity was defined “as a waist circumference of ≥ 90 cm in men or ≥ 80 cm in women” by Misra A et.al, 2009 (8).

Personal history: Demographic characteristics like Age, sex and level of education was noted and personal history with emphasis on physical activity, tobacco use, alcohol consumption and known history of diabetes mellitus, hypertension, and hypercholesterolemia was recorded.

4.9 Measurement of physical anthropometry parameters:

4.9.1 Physical activity: Physical activity was expressed as “metabolic equivalents (METS) and subjects were classified into sedentary and non-sedentary, with those reporting physical activity for at least 30 min a day for a minimum of 5 days a week (household activities involving physical effort, walking to and from work involving at least 30 min., manual workers, those performing leisure-time physical activity) as non-sedentary and all others were classified as sedentary” as given by WHO,2010 (9).

4.9.2 Measurement waist to hip ratio (WHR):

It was calculated by measuring waist at narrowest point under lowest rib (WC=waist circumference) and hips at the widest portion of buttocks (HC=hip circumference) using a standard measuring tape and the ratio WC (in cm)/ HC (in cm).

4.9.3 Measurement body mass index (BMI):

Subjects' height was measured to the nearest centimeter using a wall-mounted stadiometer (Model 206, Seca, Hamburg Germany), with the subject standing upright without shoes, with their back against the wall, heels together and eyes directed forward. Weight was measured in kilograms to the nearest 0.5Kg employing digital weighing scale which is portable and can be kept on a firm horizontal surface. Body mass index was calculated as "weight in kg/ (height in meter squared) and was categorized as normal ($< 25 \text{ kg/m}^2$), overweight ($25\text{--}30 \text{ kg/m}^2$) and obese ($>30 \text{ kg/m}^2$)" as noted by (World Health Organisation, 2008) (10).

Three readings of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded with mercury sphygmomanometer. Data was analyzed by taking the average of the last two readings. Heart rate (bpm) of all the subjects was also recorded. Trained technicians performed the resting 12 lead electrocardiograms (ECG) on all the enrolled subjects with digital ECG recorders which display the measured parameters. Minnesota coding for five consecutive complexes were recorded for each lead (11). The CAD patients were followed for a month to document the MACEs in them.

4.10 Biochemical parameters:

Five mL fasting venous blood was drawn from cubital vein on the day of admission. One part of blood sample (2ml) was collected in an EDTA vial and was used for estimation of glycosylated hemoglobin (HbA1c). The serum was used for estimation of fasting blood glucose (FBG), serum lipids, blood urea nitrogen (BUN), and creatinine by commercial laboratory methods according to the manufacturer's instruction which are a part of the management protocol for CAD. The serum was separated and were stored at -80°C until assayed and the remaining sample shall be used for estimation of Serum levels of hs-CRP, and $\text{TNF-}\alpha$ as inflammatory

biomarkers and IL-10 levels as the anti- inflammatory biomarker by the method of Enzyme Linked Immunosorbent Assay.

Table 4.1: List of biochemical tests, principle and instrument used in the study:

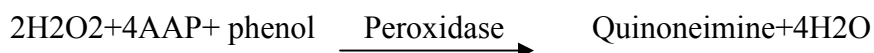
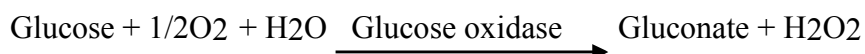
S N	Test	Method	Instrument
1	FBG mg/dL	Glucose oxidaseand peroxidase	Ortho Clinical Diagnostics VITROS 5,1 FS fully automated chemistry analyzer
2	HbA _{1c} %	HPLC	Bio-Rad, D-10
3	Total Cholesterol mg/dL	Cholesterol oxidase and peroxidase	Ortho Clinical Diagnostics VITROS 5,1 FS fully automated chemistry analyzer
4	Triglyceride mg/dL	Trinder method	Ortho Clinical Diagnostics VITROS 5,1 FS fully automated chemistry analyzer
5	HDL-C mg/dL	Direct method	Ortho Clinical Diagnostics VITROS 5,1 FS fully automated chemistry analyzer
6	Blood urea nitrogen (BUN) mg/dL	Urease-based enzymatic reaction	Ortho Clinical Diagnostics VITROS 5,1 FS fully automated chemistry analyzer
7	Creatinine mg/dL	Enzyme based Jaffe reaction.	Ortho Clinical Diagnostics VITROS 5,1 FS fully automated chemistry analyzer
8	hsCRP mg/L	ELISA	Calbiotech Batch CR56381
9	TNF-alpha pg/mL	ELISA	Calbiotech Batch 1100130
10	IL-10 pg/mL	ELISA	Calbiotech Batch 101071

4.10.1. Estimation of blood glucose

Blood glucose was estimated in Ortho Clinical Diagnostics VITROS 5,1 FS fully automated chemistry analyzer by glucose oxidase peroxidase method an end point colorimetric method.

Principle:

“Glucose is determined by enzymatic oxidation method in the presence of glucose oxidase (GOD) and peroxidase method. The hydrogen peroxide thus formed reacts with phenol and 4- aminoantipyrine catalysed by peroxidase to form a red coloured quinoneimine compound, color produced is directly proportional to the amount of glucose present in the given sample. Optical density was measured at 500 nm” as given by (P Trinder 1969) (12).



Reagents:

Reagent 1: Glucose mono reagent

Phosphate buffer (pH 7.5)	= 200 mmol/L
Glucose oxidase	≥ 20000 U/L
4 AAP	= 0.3 mmol/L
Phenol	= 10 mmol/L
Peroxidase	≥ 2000 U/L Reagent

2: Glucose standard 100 mg/dL

Assay

Mode : End point method Wavelength : 500 nm (480-520nm)

Optical path : 1 cm Temperature: 37°C

Measurement against blank sample

Procedure:

Reagents	Blank	Standard	Test
Serum	-	-	10 μ L
Standard	-	10 μ L	-
Glucose reagent	1000 μ L	1000 μ L	1000 μ L

Mix well and incubate at 37⁰C for 10 minutes. Measure the absorbance of standard and test against blank at 500nm within one hour.

Calculation

$$\text{Concentration of Glucose (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

Reference values

Plasma glucose (fasting) = 70-110 mg/dL

4.10.2 Estimation of glycosylated hemoglobin (HbA1c) by high performance liquid chromatography (HPLC) method (13)

Glycemic status of diabetic patients was assessed by HbA1c levels. It was measured by commercially available kit from Bio-Rad by HPLC method.

Principle: Glycosylated hemoglobin was estimated based on the principle of chromatographic separation of the analyte by ion-exchange high performance liquid chromatography method.

Kit components

Elution Buffer: Two bottles containing 2000mL of a Bis-Tris phosphate buffer, pH 6. Contains <0,05% sodium azide as a preservative.

Elution Buffer: One bottle containing 1000mL of a Bis-Tris phosphate buffer, pH 6.7. Contains <0,05% sodium azide as a preservative.

Wash/Diluent set: One bottle containing 1600 mL of deionized water with <0,05% sodium azide as a preservative.

Analytical cartridge: One cation exchange cartridge 4.0 ID X 30mm

HbA1c Calibrator: Consist of 3 vials of calibrator level 1, 3 vials of calibrator level 2 and bottle of calibrator diluents.

Specimen type: Whole blood

Preservative: The whole blood specimen should be collected in a vacuum collection tube containing EDTA

Storage: Stored for one day at room temperature i.e 15 to 30⁰ C or upto 4 days at 2-8⁰ C. Specimen preparation: Ensuring that the sample barcodes are facing the back of the instrument, sample tubes were loaded into the D-10 sample rack and placed in it. If the sample is less than 2 mL then it should be prediluted.

Procedure:

The samples are diluted automatically in the D-10 instrument and injected into the analytical cartridge. The D-10 delivers buffer to the cartridge on gradient basis with increasing the ionic strength. Then hemoglobin variants are separated depending on their ionic interaction with the cartridge. Later these separated hemoglobin pass through the flow cell photometer. The absorbance is measured at wavelength of 415 nm.

Interpretation of the results: Reportable range – 3.8 -18.5 %Reference values:

4-5.6%	Normal range
5.7 – 6.5%	High risk of developing DM
>6.5%	Diabetes mellitus

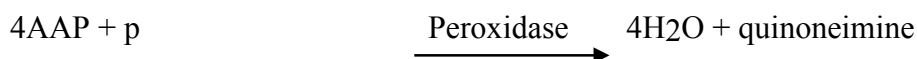
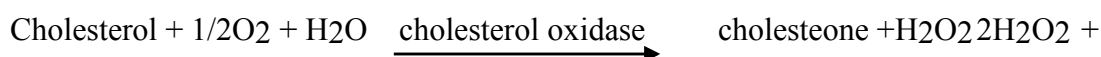
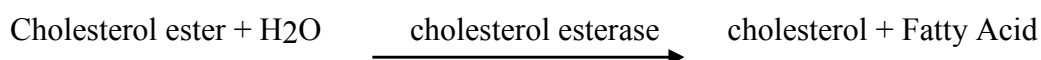
4.10.3 Estimation of total cholesterol (14)

Lipid profile includes total cholesterol, triglyceride and high density lipoprotein. These were measured in in Ortho Clinical Diagnostics VITROS 5,1 FS fully automated chemistry analyzer. VLDL-C and LDL-C was calculated.

Method: Cholesterol oxidase and peroxidase

Principle:

The free and esterified cholesterol in the given sample is determined by enzymatic method



Absorbance of quinoneimine formed is directly proportional to the concentration of cholesterol in the given sample and absorbance measured at 480 to 520 nm of wavelength.

Reagents:

I..Cholesterol reagent composition:

Sodium cholate: 0.5 mmol/L

4-Aminoantipyrine (4AAP): 0.5 mmol/L

Enzymes:

Cholesterol esterase: ≥ 0.2 U/mL

Cholesterol oxidase: ≥ 0.1 U/mL

Peroxidase: ≥ 0.8 U/mL

II. Calibrator/ Cholesterol Standard

Cholesterol Concentration: 200 mg/dL

III. Assay Parameters

Mode: End-point method

Wavelength: 500 nm

Optical Path Length: 1 cm

Procedure:

Reagents	Blank	Standard	Test
Serum	-	-	10 μ L
Standard	-	10 μ L	-
Cholesterol reagent	1000 μ L	1000 μ L	1000 μ L

Mix well. Incubate all the tubes for 10 minutes at room temperature or at 37⁰C for 5 minutes.

Calculation: Absorbance of test*20/ Absorbance of standard

Reference range: (As per NCEP ATP III guidelines,1974)(7)

For Total cholesterol

Desirable : < 200mg/dL

Borderline high : 200-239mg/Dl

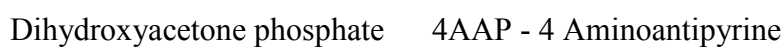
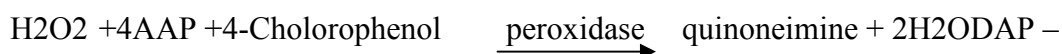
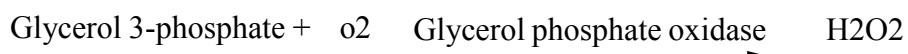
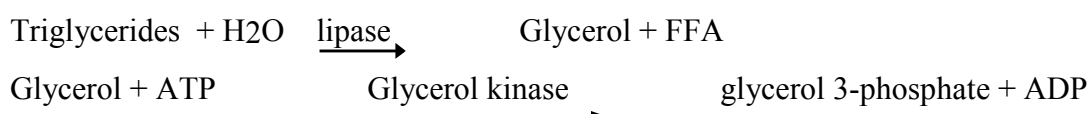
High : $\geq$ 240mg/dL

4.10.4 Estimation of Triglycerides (15)

Method: By “Glycerol phosphate oxidase/ peroxidase method”.

Principle:

“Triglycerides in the blood sample can be measured by spectrophotometrically. The intensity of color formed is directly proportional to the triglyceride concentration in the sample when measured at 500nm” as given by (Fossati P et.al,1982) (15)



Reagents:

Triglyceride mono reagent:

Pipes buffer - 45 mmol/L

4-chlorophenol - 6 mmol/L

Magnesium chloride - 5 mmol/L

ATP - 1 mmol/L

Lipase > 100 U/mL

Peroxiadse ≥ 0.8 U/mL

Glycerol kinase ≥ 1.5 U/mL

4 AAP - 0.75mmol/L

Glycerol 3 phosphate oxidase $\geq$ 4 U/mL

Triglyceride standard:

Assay:

Mode : End point

Wavelength : 500nm

Optical path length : 1 cm

Procedure:

	Blank	Standard	Test
Serum	-	-	10 μ L
Standard	-	10 μ L	-
Reagent	1000 μ L	1000 μ L	1000 μ L

Mix well. Incubate at tubes for 15 minutes at room temperature for 5 minutes.

Calculation:

$$\text{Triglyceride concentration (mg/dL)} = \frac{\text{Absorbance of test} \times 200}{\text{Absorbance of standard}}$$

Reference range: (as per NCEP ATP III guidelines)(7)

Normal : < 150 mg/dL

Borderline high : 150-199 mg/dL

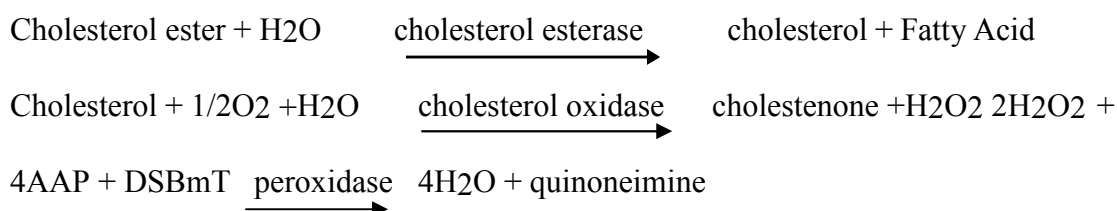
High : 200-499 mg/dL

Very high : $\geq$ 500 mg/dL

4.10.5 Estimation of HDL Cholesterol (16)

Principle:

“The cholesterol from LDL-C, VLDL-C and chylomicrons, is broken down by the cholesterol oxidase in an enzymatic reaction. The detergent in reagent B, will solubilizes cholesterol from HDL-C in the given sample. The HDL-C is measured at 600 to 700 nm. Absorbance of quinoneimine formed is directly proportional to amount of HDL-C present in the given sample.” As given by Warnick GR et al, 2001(16).



Reagents:

1. Reagent:

Cholesterol oxidase	<	1U/mL
Peroxidase	<	1U/mL
N,N-bis(4-sulfobutyl)-m-toluidine (DSBmT) - Accelerator	-	1mmol/L

2. Reagent:

Cholesterol esterase	<	1.5 U/mL
4-aminoantipyrine	-	1 mmol/L
Ascorbate oxidase	<	3KU/L

Assay:

Mode	:	End point
Wavelength	:	600-700nm
Optical path length	:	1cm

Procedure:

Pipette into tube marked	Test
Serum	7 µL
Reagent A	750 µL
Mix well and insert the cuvette in the photometer. After 5 minutes read the absorbance (A1) at 600/700 nm against distilled water	
Reagent B	250µL
Mix well. Incubate at 37 ⁰ C for 5 minutes and read the absorbance (A2) at 600/700 nm.	

Calculation:

$$\text{Concentration of HDL} = \frac{\text{Absorbance of (A2-A1) sample}}{\text{Absorbance of calibrator (A2-A1)}} \times \text{Calibrator}$$

Reference range: (As per NCEP ATP III guidelines) (7)

For HDL cholesterol

Low risk : ≥ 60 mg/dL

High risk : < 35 mg/dL

4.10.6 - Estimation of LDL cholesterol (17)

“Low density lipoprotein was calculated using “Friedwald’s equation” and was applied for those TG levels which were less than 400 mg/dL.

LDL-C = Total cholesterol – Triglycerides - HDL-C” as given by (Friedwald WT et al, 1972)(17)

5

Reference range: As per NCEP ATP III Guidelines (7)

Optimal	< 100mg/dL
Near optimal	: 100-129mg/dL
Borderline high	: 130-159mg/dL
High	: 160-189mg/dL
Very high	≥190mg/dL

4.10.7- Estimation of High sensitive C-reactive protein (18)

Principle: C-reactive protein (CRP) is a pentameric acute-phase reactant synthesized primarily by the liver in response to inflammatory stimuli, particularly interleukin-6 (IL-6). As a key component of the innate immune system, CRP plays a role in the body's initial response to infection, tissue injury, and inflammation. Its concentration in the bloodstream can increase dramatically, often by several hundred-fold, within hours of an inflammatory insult, peaking at 24-48 hours. This rapid and substantial rise makes CRP a valuable general biomarker for systemic inflammation. The estimation of high-sensitivity C - reactive protein (hsCRP) by Enzyme-Linked Immunosorbent Assay (ELISA) is based on a sandwich type solid-phase enzyme immunoassay. An anti-human CRP coating antibody was adsorbed onto microwells. Any hsCRP present in the sample binds to the immobilized capture antibody. After an incubation period, unbound components are removed by washing. Subsequently, a horseradish peroxidase (HRP)-conjugated detection antibody, also specific for human CRP, is added to the wells. This detection antibody binds to a different epitope of the captured hsCRP, forming an antibody-antigen-antibody sandwich complex.

Following a second incubation and washing step to remove excess enzyme conjugate, a chromogenic substrate solution (e.g., Tetramethylbenzidine, TMB) is added. The HRP enzyme catalyzes the conversion of the colorless substrate into a colored product. The intensity of the color developed is directly proportional to the concentration of hsCRP present in the original sample. The reaction is stopped by adding an acidic stop solution, which also enhances the color stability. The absorbance of the colored product is then measured spectrophotometrically at a specific wavelength (typically 450 nm, with an optional reference wavelength of 620 nm or 630 nm). A standard curve generated from known concentrations of hsCRP is used to determine the hsCRP concentration in Patient samples.

Preparation of the Reagents:

A. Standards and Controls: The lyophilized standards of known concentrations (e.g., 0, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 mg/L) and controls to the volume specified on the vial label with specified volume of standard diluent /diluent were

reconstituted. They were allowed to remain undisturbed until completely dissolved, and then mix well by gentle shaking.

B. Wash Solution: 12.5 ml of washing solution concentrate in 250 ml distilled water was diluted to prepare a 1X working wash buffer with proper mixing which could be stored at 2–8°C for up to one week.

C. Substrate Solution (TMB): Ready-to-use.

D. **Stop Solution:** Ready-to-use acidic solution (e.g., 0.5 M H₂SO₄ or 1 M HCl)

Assay Procedure:

1. The required number of strips was selected for the run. The unused strips were resealed in the bag with desiccant and stored at -80 °C.
2. The strips were secured into the holding frame.
3. Placed microwell strips containing the standard curve in position A1/A2 to H1/H2.
4. 100 µl of distilled water was added to the sample wells.
5. Distilled water was also added to all standard and blank wells as indicated on the label of the standard strips (A1, A2 to H1, H2).
6. 50 µl of each sample was added in duplicate (diluted with the diluent 1:200 as the levels of hsCRP expected were in the lower range), into the designated wells and the content was mixed properly.
7. Microtiter plate was covered with an adhesive film and incubated at room temperature (18°C to 25°C) for 60 minutes.
8. Adhesive film was removed and the microwell strips were washed 6 times with approximately 300 µl Wash Buffer per well with thorough aspiration of microwell contents between washes. Wash Buffer was allowed to sit in the wells for about 10 – 15 seconds before aspiration. After the last wash, microwell strips were tapped on absorbent pad or paper towel to remove excess Wash Buffer.
9. Add 100 µL of the prepared HRP-conjugated detection antibody to each well. Cover the plate with a fresh adhesive plate sealer. Incubate the plate at room temperature (20–25°C) for the specified time - 30 minutes.
10. Adhesive film was removed and the microwell strips were washed 4 times as in step 8.

11. 100 µl of TMB Substrate Solution was added to all wells, including the blank wells.
12. The plate was incubated for 15 min. at room temperature on a horizontal shaker set at 700 rpm $\pm$ 100 rpm, avoiding direct sunlight.
13. 100µl of Stop Solution was pipetted into each well.
14. The absorbance was taken at 450 nm as the primary wave length (optionally 620 nm as the reference wavelength, the absorbance of both the samples and the human hsCRP standards were determined.

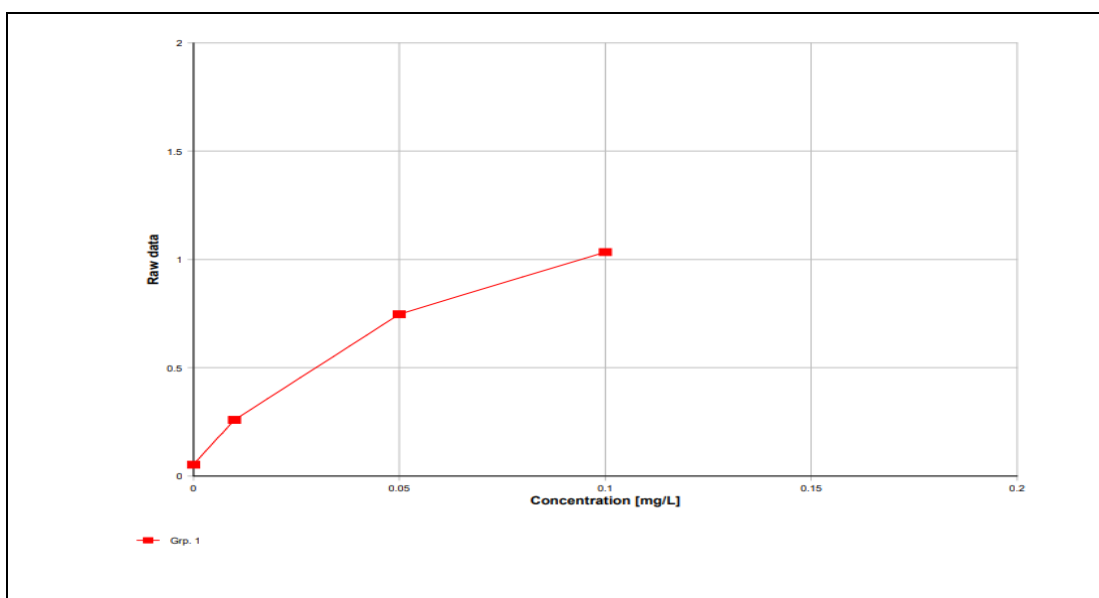
Calculation of Analytical Results:

A standard curve was constructed using all the standard points for which absorbances are below the limit of linearity of reader used.

The OD on the ordinate against the standard concentrations was plotted on the abscissa using either linear or semi-log paper and the curve was drawn by connecting the plotted points with straight lines. The HsCRP concentration of samples was determined for which absorbance was not greater than those of the last standard plotted at 450nm.

$$\text{Actual hsCRP concentration (mg/L)} = \text{Concentration from curve (mg/L)} \times \text{Dilution Factor}$$

Figure 4.2: Standard curve for hsCRP (mg/l) estimation.



4.10.8: ESTIMATION OF SERUM INTERLEUKIN-10 (IL-10) {Calbiotech}: (19)

Principle: Interleukin-10 is a pleiotropic cytokine playing an important role as a regulator of lymphoid and myeloid cell function. An anti-human IL-10 coating antibody was adsorbed onto microwells. Human IL-10 present in the sample to antibodies adsorbed to the microwells; a biotin-conjugated anti-human IL-10 antibody binds to human IL-10 captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated antihuman IL-10. Following incubation unbound biotin conjugated antihuman IL-10 and Streptavidin- HRP was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A coloured product was formed in proportion to the amount of soluble human IL-10 present in the sample. Bound enzyme-labeled antibodies were measured through a chromogenic reaction. The reaction was terminated by addition of acid and absorbance was measured at 450 nm. The amount of substrate turnover was determined calorimetrically by measuring the absorbance, which was proportional to the IL-10 concentration. A standard curve was prepared from 7 human IL-10 standard dilutions and human IL-10 sample concentration determined.

Preparation of The Reagents:

A. Standards and Controls: The lyophilized standards and controls to the volume specified on the vial label with distilled water were reconstituted. They were allowed to remain undisturbed until completely dissolved, and then mix well by gentle shaking.

B. Wash Solution: 12.5 ml of washing solution concentrate in 250 ml distilled water was diluted.

Assay Procedure:

1. The required number of strips was selected for the run. The unused strips was resealed in the bag with desiccant and stored at -80 C.
2. The strips were secured into the holding frame.
3. Placed microwell strips containing the standard curve in position A1/A2 to H1/H2.
4. 100 µl of distilled water was added to the sample wells.
5. Distilled water was also added to all standard and blank wells as indicated on the label of the standard strips (A1, A2 to H1, H2).

-
6. 50 μ l of each sample was added in duplicate, in to the designated wells and the content was mixed properly.
 7. Microtiter plate was covered with an adhesive film and incubated at room temperature (18°C to 25°C) for 3 hours.
 8. Adhesive film was removed and the microwell strips was washed 6 times with approximately 300 μ l Wash Buffer per well with thorough aspiration of microwell contents between washes. Wash Buffer was allowed to sit in the wells for about 10 – 15 seconds before aspiration. After the last wash, microwell strips were tapped on absorbent pad or paper towel to remove excess Wash Buffer.
 9. 100 μ l of TMB Substrate Solution was added to all wells, including the blank wells.
 10. The plate was incubated for 15 min. at room temperature on a horizontal shaker set at 700 rpm $\pm$ 100 rpm, avoiding direct sunlight.
 11. 100 μ l of Stop Solution was pipetted into each well.
 12. The absorbance was taken at 450 nm as the primary wave length (optionally 620 nm as the reference wavelength, the absorbance of both the samples and the human IL-10 standards were determined.

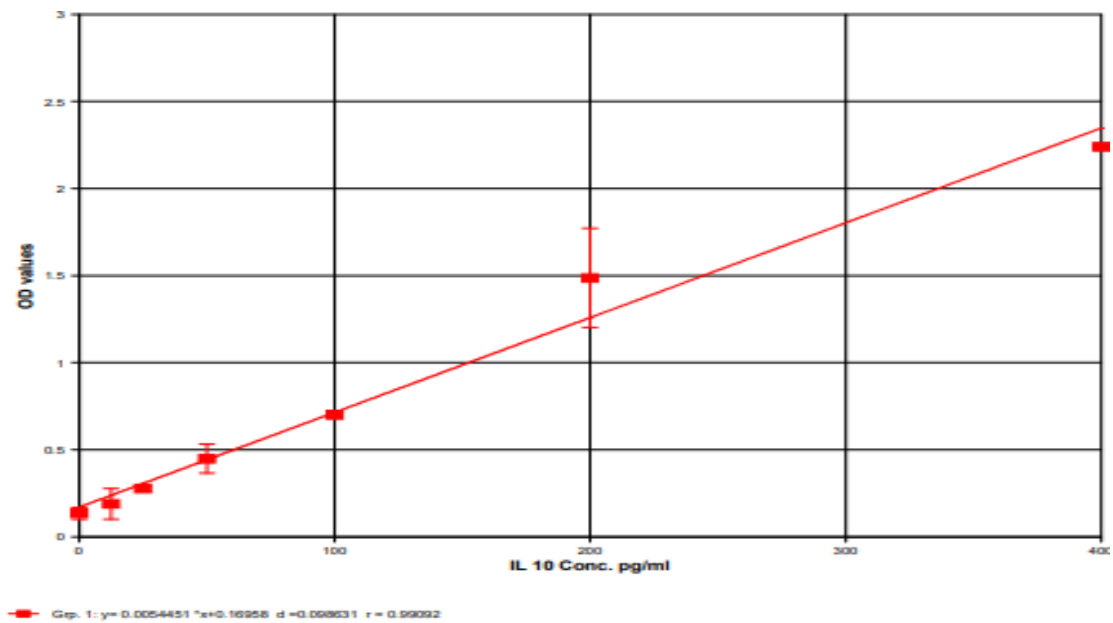
Calculation of Analytical Results:

A standard curve was constructed using all the standard points for which absorbances are below the limit of linearity of reader used.

The OD on the ordinate against the standard concentrations was plotted on the abscissa using either linear or semi-log paper and the curve was drawn by connecting the plotted points with straight lines.

The IL-10 concentration of samples was determined for which absorbance was not greater than those of the last standard plotted at 450nm.

Figure 4.3: Standard curve for IL-10(pg/ml) estimation.



4.10.9 Estimation of TNF-alpha levels (20)

The commercially available human TNF- α ELISA kit (Calbiotech) 9, was used to determine the concentration of serum TNF- α . This kit measures TNF- α in serum, plasma, cell supernatants or buffered solutions.

Principle:

Principle: Tumor Necrosis Factor- α (TNF- α), also known as cachectin, is a polypeptide cytokine produced by monocytes and macrophages. It functions as a multipotent modulator of immune response and further acts as a potent pyrogen. The human TNF- α Instant ELISA was an enzyme-linked immunosorbent assay for the quantitative detection of human TNF- α . An anti-human TNF- α coating antibody was adsorbed onto microwells. Human TNF- α present in the sample to antibodies adsorbed to the microwells; a biotinconjugated anti-human TNF- α antibody binds to human TNF- α captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-human TNF- α . Following incubation unbound biotin conjugated antihuman TNF- α and Streptavidin- HRP was removed during a wash step, and substrate solution reactive with HRP was added to the wells. A coloured product was formed in proportion to the amount of soluble human TNF- α present in the sample. Bound enzyme-labeled antibodies were measured through a chromogenic reaction. The reaction was terminated by addition of acid and absorbance is measured at 450

nm. The amount of substrate turnover was determined calorimetrically by measuring the absorbance, which was proportional to the TNF- α concentration. A standard curve was prepared from seven human TNF- α standard dilutions and human TNF- α sample concentration determined.

Preparation of The Reagents:

A. Standards and controls: The lyophilized Standards and Controls to the volume specified on the vial label with distilled water were reconstituted. They were allowed to remain undisturbed until completely dissolved, and then mix well by gentle shaking.

B. Wash Solution: 12.5 ml of washing solution concentrate in 250 ml distilled water was diluted.

Assay Procedure:

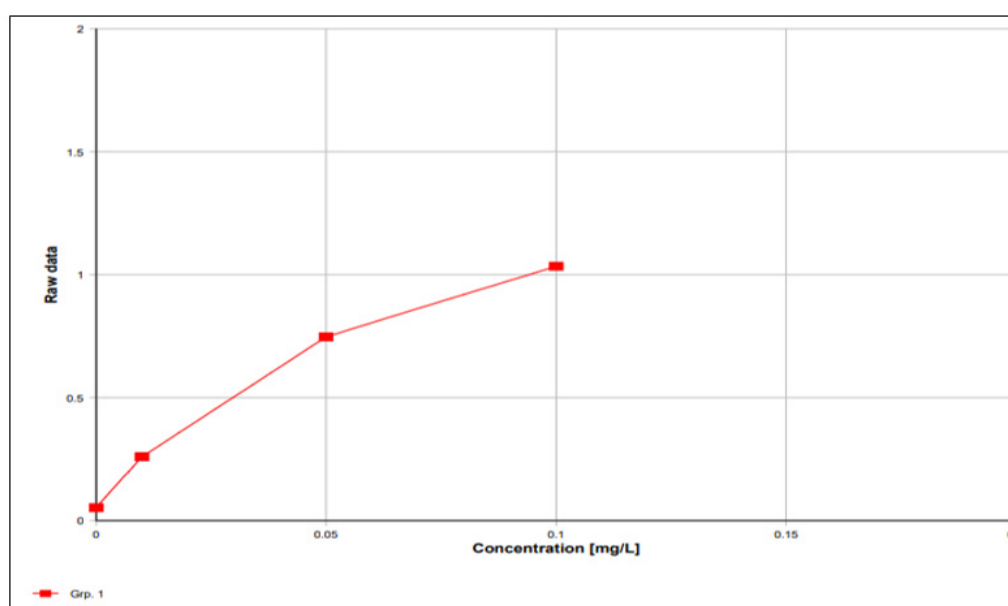
1. The required number of strips was selected for the run. The unused strips was resealed in the bag with desiccant and stored at -80 C.
2. The strips were secured into the holding frame.
3. Placed microwell strips containing the standard curve in position A1/A2 to H1/H2.
4. 100 μ l of distilled water was added to the sample wells.
5. Distilled water was also added to all standard and blank wells as indicated on the label of the standard strips (A1, A2 to H1, H2).
6. 50 μ l of each sample was added in duplicate, in to the designated wells and the content was mixed properly.
7. Microtiter plate was covered with an adhesive film and incubated at room temperature (18°C to 25°C) for 3 hours.
8. Adhesive film was removed and the microwell strips was washed 6 times with approximately 300 μ l Wash Buffer per well with thorough aspiration of microwell contents between washes. Wash Buffer was allowed to sit in the wells for about 10 – 15 seconds before aspiration. After the last wash, microwell strips were tapped on absorbent pad or paper towel to remove excess Wash Buffer.
9. 100 μ l of TMB Substrate Solution was added to all wells, including the blank wells.
10. The plate was incubated for 15 min. at room temperature on an on a horizontal shaker set at 700 rpm $\pm$ 100 rpm, avoiding direct sunlight.
11. 100 μ l of Stop Solution was pipetted into each well.

12. The absorbance was taken at 450 nm as the primary wave length (optionally 620 nm as the reference wavelength, the absorbance of both the samples and the human TNF- α standards were determined.

Calculation of Analytical Results:

- A standard curve was constructed using all the standard points for which absorbances were below the limit of linearity of reader used.
- The OD on the ordinate against the standard concentrations was plotted on the abscissa using either linear or semi-log paper and the curve was drawn by connecting the plotted points with straight lines.

Figure 4.4: Standard curve for TNF-alpha (mg/l) estimation.



The TNF-alfa concentrations of samples and controls was determined for which absorbance was not greater than those of the last standard plotted at 450nm.

4.11 Dietary intake and Calculation of Dietary Inflammatory Index

The Food Frequency Questionnaire-NK was framed for use in the north Karnataka food population. It was developed and validated for use in the present study by the Principal Investigator. It was used to adequately capture the average dietary intakes of the research subjects to calculate the DII score (21). Frequency of each item was derived as (2-3 times per day, 1 time per day, 3-4 times per week, 1-2 times per week,

2-3 times per month, 1 time per month or less, Rarely/never). The noted frequency for each food item was then converted to a daily intake using household measures to ascertain approximate portion sizes in grams. The above details were then used to calculate the DII to assess the pro-inflammatory or anti-inflammatory potential of the diet (22). Construct validation of DII are described in previous studies (23-26).

Briefly, dietary data from each study subject is linked to a regionally representative global database. This database denotes a potent estimate of means and standard deviations for each of the food parameters considered (i.e. all nutrients of food). (22). A 'z' score for each of the food parameters will be calculated using the FFQ-derived dietary information for each subject. 'Standard global mean' will be subtracted from individuals' intake of each dietary parameter and then this value was divided by the world standard deviation (SD). To minimize the effect of 'right skewing'- feature which is common with dietary data, this value was then converted to a centered percentile score. The centered percentile value was multiplied by the respective inflammatory effect scores derived from a literature review and scoring of 1943 'qualified' articles, to obtain the subject's food parameter-specific DII score. All of the food parameter-specific DII scores were then summed to create the overall DII score for each subject in the study. The greater the DII score, the more pro-inflammatory the diet was considered; negative values indicated the anti-inflammatory nature of the diet. The range of the DII score is from -8.87 (maximally anti-inflammatory) and +7.98 (maximally pro-inflammatory). DII scores were then be converted to quartiles (Quartile 1: -6.27--1.26; Quartile 2: -1.25-0.74; Quartile 3: 0.74-2.63; and Quartile 4: 2.64-5.89) for the convenience of statistical application.

The following food parameters were used in the DII calculation as pro-inflammatory: total calories as energy, carbohydrates, and total fat (saturated as well as cholesterol); whereas, protein, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3-fatty acids, n-6 fatty acids, fiber, iron, vitamin A, β -carotene, thiamin, riboflavin, niacin, vitamin C, garlic, ginger, onion, tea, and pepper were valued as anti-inflammatory. Parameters that were not obtained due to an incomplete food database or due to very low intake were omitted during the DII calculation.

The CAD patients were followed for a month to document the major adverse cardiac events (MACEs) in them. They wer categorized on the basis of DII scores into quartiles and the data will be processed with appropriate statistical tools.

4.12 Statistical analysis:

Statistical analyses were performed using SPSS 26 (SPSS Inc., version 0.21, Chicago, IL). *p*-values less than 0.05 were considered statistically significant. The normal distribution of the variables was investigated using the Kolmogorov–Smirnov test. The values of qualitative variables were presented as percentage. The values of quantitative data were presented as mean $\pm$ standard deviation and also as medians. *For statistical ease, subjects were ranked based on DII quartiles.* Chi-square test was applied for Qualitative variables and the Quantitative variables were tested with ANOVA (one-way analysis of variance) to compare data between the DII quartiles followed by Bonferroni Correction as a post hoc test. Binary conditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI), using quartile 1 (most anti-inflammatory diet) as the reference category. Confounding factors such as age, sex, BMI, smoking, systolic blood pressure, total serum cholesterol, diabetes, physical activity were adjusted to reduce the bias.

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CHAPTER 5

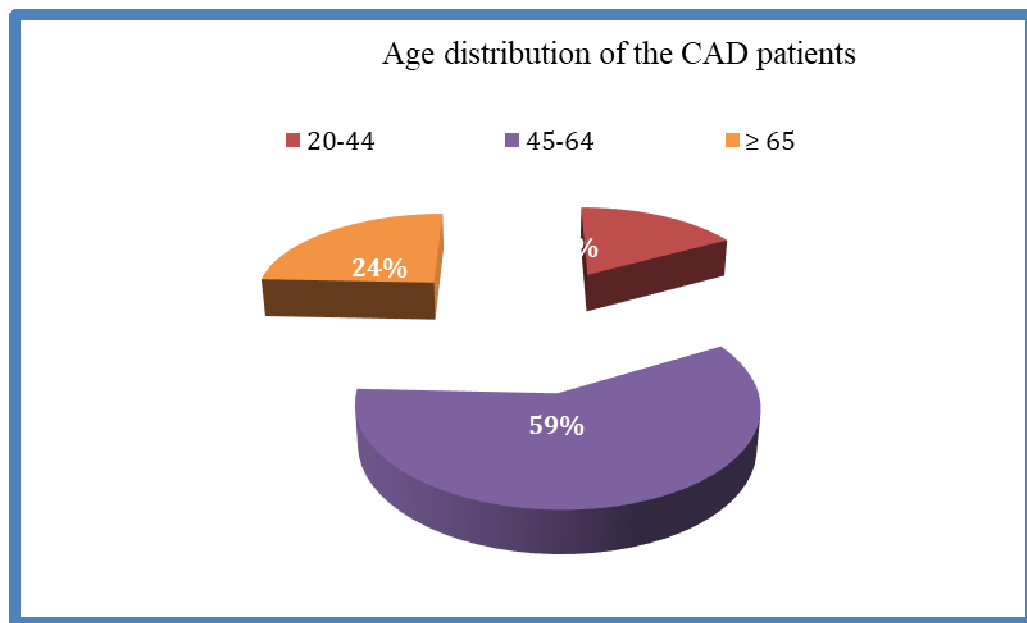
RESULTS

Results

Coronary artery disease (CAD) is the leading cause of death and morbidity worldwide. India's age-standardized CVD death rate is higher than the global average and hence this study was conducted to investigate the association of MACEs and dietary inflammation which is one of the important modulators of CAD pathogenesis.

Total 310 CAD Patients were enrolled for the study. The normality of continuous variables was assessed using the Kolmogorov-Smirnov test.

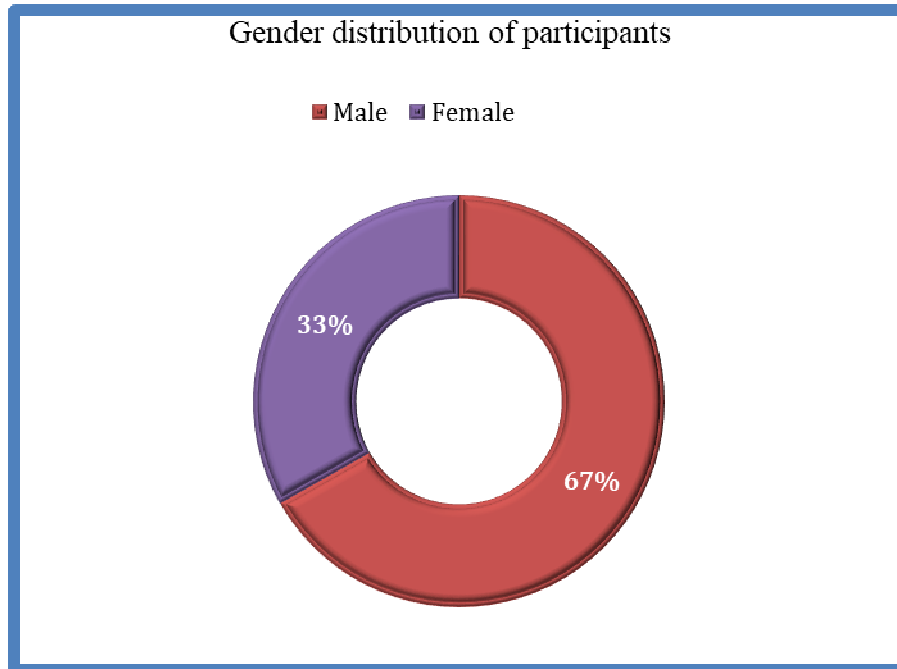
5.1-Age-wise distribution of CAD patients:



The distribution is positively skewed (since the mean is less than the median), suggesting that there are relatively younger individuals in comparison to older individuals. Majority of the participants were in the age group of 45-64 yr. 17% were between the age group of 20-44 yr. Only about 24 % were over the age of 65 yr.

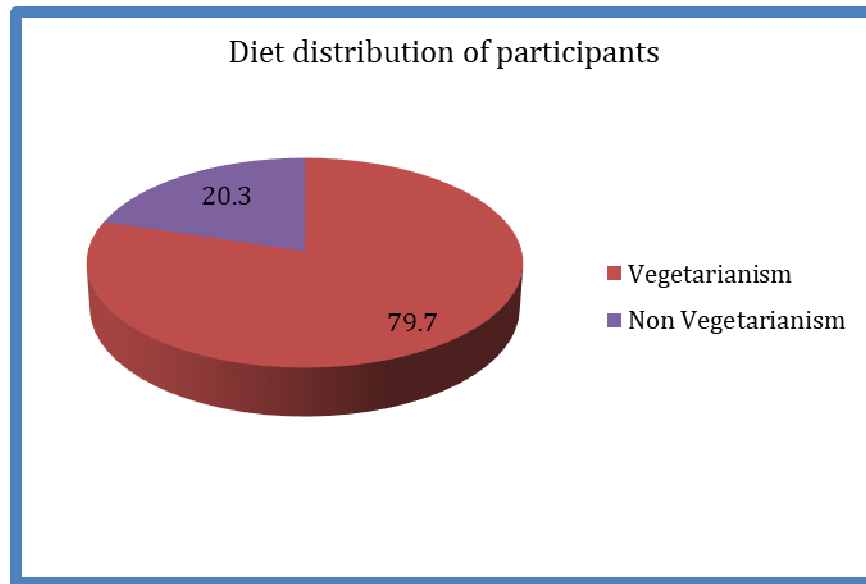
5.2 – Gender distribution of participants:

Among the participants males were more compared to females with male to female ratio of 2.03:1.



5.3- Distribution of participants on the basis of primary diet pattern

Figure 5.3: Distribution of participants in accordance to the primary diet pattern as vegetarianism and non-Vegetarianism:



Majority (79.7%) of the participants consumed a primarily vegetarian pattern of diet when compared to 20.3% who followed a diet that was predominantly non-vegetarian.

5.4 Baseline characteristics of the participants

The results are presented after employing descriptive statistics such as counts and percentages for categorical variables, and medians for continuous variables.

Table- 5.1: Baseline characteristics of the study participants

Characteristic	Range	Mean $\pm$ SD	Median
Age(yr.)	25-76	55 $\pm$ 10	56
Total household income(INR)	7000-300000	18232 $\pm$ 23668	14000
Neck circumference(cm)	30.0-37.6	33.7 $\pm$ 2.5	33.68
BMI, kg/m ²	24.2-33.9	29 $\pm$ 2.7	28.82
Waist circumference(cm)	72.0-108.2	88.6 $\pm$ 7.0	88.76
Resting HR(beats/min)	47 - 126	76.6 $\pm$ 12.5	77
SBP(mm/Hg)	100 - 180	178.1 $\pm$ 10.2	180
DBP(mm/Hg)	60 -120	80.5 $\pm$ 10.4	90
Pulse Pressure Difference	30 -180	98 $\pm$ 15.3	100
MET, min/week	0-320	117 $\pm$ 92	130
FBG (mg %)	68.3-221.6	148 $\pm$ 51	166.8
HbA1C(%)	4.-9.3	6.7 $\pm$ 1.8	7.43
Total cholesterol (mg %)	151-295	223 $\pm$ 39	222
Triglycerides (mg %)	140-542	344.7 $\pm$ 118	350
HDL-C (mg %)	36-73	53.7 $\pm$ 10	55
LDL (mg %)	112-238	179 $\pm$ 37	182
hsCRP (mg/L)	0.6-37.2	14.5 $\pm$ 7.5	12.4
TNF-alpha (pg/ml)	0.7-222.5	47.5 $\pm$ 36.7	39.9
IL-10 (pg/ml)	2.98-165.2	33.4 $\pm$ 25.3	23.2
Gensini score	0-174	23.4 $\pm$ 26.0	15
DII score	-2.0-6.3	2.28 $\pm$ 1.75	2.30

The baseline demographic, anthropometric, clinical, biochemical, inflammatory, and dietary characteristics of the study participants (n=310) are comprehensively presented in Table 1. The cohort exhibited a mean age of 55 ± 10 years, with ages ranging from 25 to 76 years. The average total household income was INR $18,232 \pm 23,668$. Anthropometric measurements indicated a mean BMI of 29 ± 2.7 kg/m², with a range from 24.2 to 33.9 kg/m², and a mean waist circumference of 88.6 ± 7.0 cm. Regarding clinical parameters, the mean resting heart rate was 76.6 ± 12.5 beats/min. The mean systolic blood pressure (SBP) was 178.1 ± 10.2 mm/Hg and diastolic blood pressure (DBP) was 80.5 ± 10.4 mm/Hg, leading to a mean pulse pressure difference of 98 ± 15.3 mm/Hg. Physical activity, assessed by Metabolic Equivalent of Task (MET), averaged 117 ± 92 min/week. Biochemical markers revealed a mean fasting blood glucose (FBG) of 148 ± 51 mg% and an average HbA1c of $6.7 \pm 1.8\%$. Lipid profiles showed mean total cholesterol of 223 ± 39 mg% and triglycerides as 344.7 ± 118 mg%, HDL-C at 53.7 ± 10 mg% and LDL at 179 ± 37 mg%. Importantly, inflammatory markers were also evaluated. The mean hsCRP level was 14.5 ± 7.5 mg/L, ranging from 0.6 to 37.2 mg/L, Mean TNF-alpha was 47.5 ± 36.7 pg/ml, while mean IL-10 was 33.4 ± 25.3 pg/ml. The mean Gensini score, an indicator of coronary artery disease severity, was 23.4 ± 26.0 , spanning from 0 to 174. Finally, the dietary inflammatory index (DII) score averaged 2.28 ± 1.75 , indicating a predominantly pro-inflammatory dietary pattern within the cohort.

5.5 -Established cardiovascular risk factors in CAD

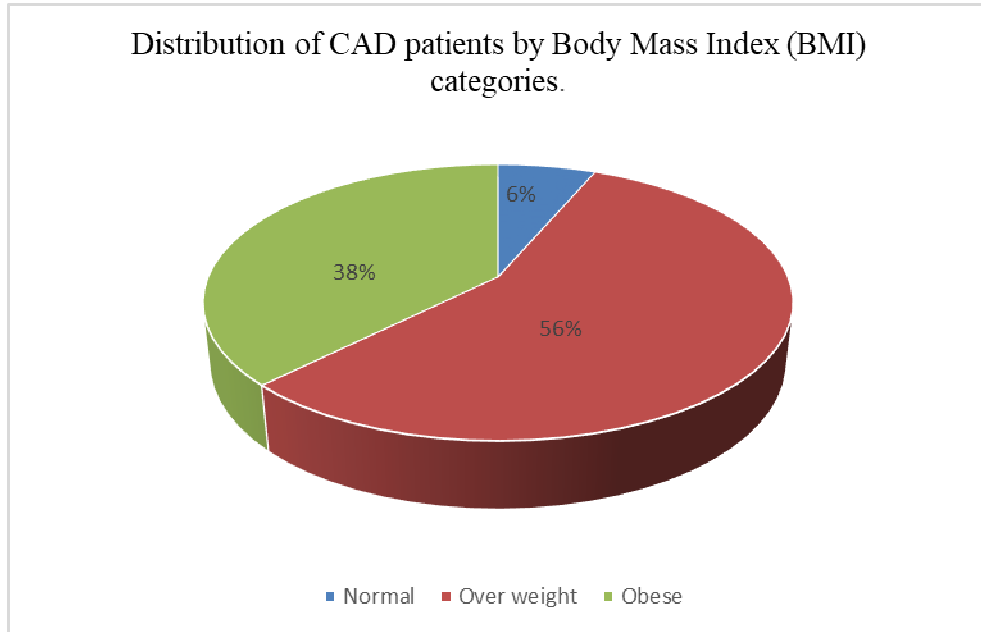
Table- 5.2: Established cardiovascular risk factors in the participants at the baseline.

Parameter	Frequency (%)
BMI	
Normal	20 (6.4%)
Over weight	174 (56.1%)
Obese	116 (37.5%)
Waist circumference(cm)	
Low risk	149(48.2%)
High risk	112 (36.2%)
Very high risk	48 (15.6%)
HTN	159(51.8%)
Dyslipidemia	256(82.7%)
DM	169(54.5%)
Tobacco	77(24.6%)
Smoking	165(53.2%)
Physical Inactivity	102(32.5%)

Table 5.5 outlines the frequency and prevalence of established cardiovascular risk factors among the study participants. A substantial proportion of the cohort were **overweight** (56.1%, n=174) or **obese**

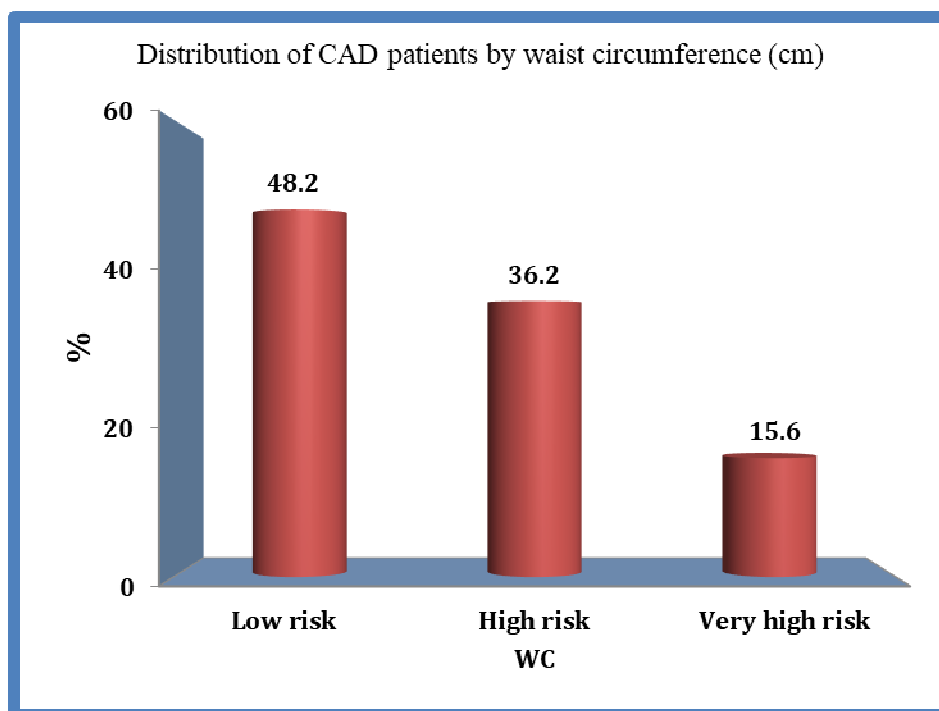
Similarly, **waist circumference** indicated varying levels of risk. Nearly half of the participants (48.2%, n=149) were categorized as low risk, while a significant portion fell into high risk (36.2%, n=112) and very high risk (15.6%, n=48) categories. Regarding other key risk factors, over half of the participants presented with **hypertension** (51.8%, n=159), **diabetes mellitus** (54.5%, n=169), and **smoking** (53.2%, n=165). The most prevalent risk factor observed was **dyslipidemia**, affecting a large majority of the cohort (82.7%, n=256). Additionally, **physical inactivity** was noted in 32.5% (n=102) of participants, and **tobacco use** (encompassing chewing forms) was present in 24.6% (n=77) of the population.

Figure 5.4– Distribution of CAD patients by Body Mass Index (BMI) categories.



Only 6% of the participants were normal on BMI, majority of them were overweight (56%), and 38% were classified as obese according to the BMI. BMI is a crucial modifiable risk factor for CAD, and its distribution within this patient group provides insight into their baseline cardiometabolic status.

Figure 5.5– Distribution of CAD patients by waist circumference (cm).



Majority (48.2%) of the CAD patients were classified as low risk, 36.2% were with high risk and 15.6% were classified as very high risk sub set. This anthropometric measure provides crucial insight into central obesity, a well-established modifiable risk factor for CAD.

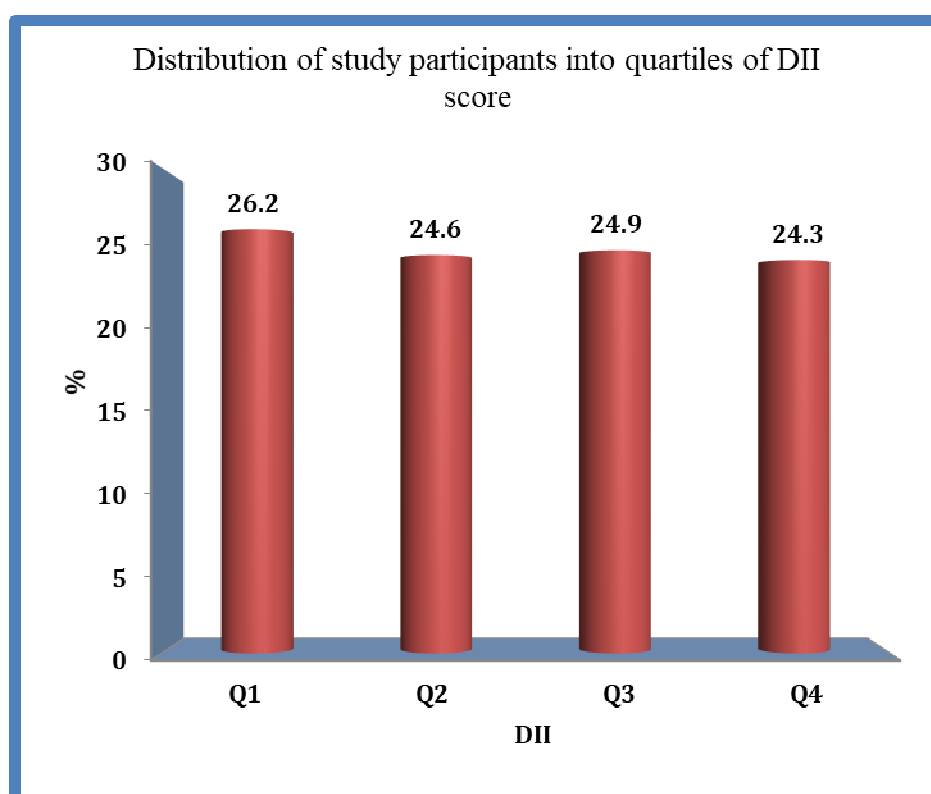
5.6-Dietary Inflammatory Index (DII) in the CAD patients primarily consuming north Karnataka food pattern.

In our study, The DII score ranged between -2.01 to +6.38. The mean $\pm$ SD of the DII score of the study participants was 2.28 ± 1.75 .

5.6.1: Classifying the CAD participants on the basis of DII quartiles.

DII scores were then converted to quartiles (Q 1: -2.01 to +1.24; Q 2: +1.25 to 2.34; Q 3: 2.35 to 3.24; and Q 4: 3.25 to 6.38) for the convenience of statistical application. The study participants were grouped from Q1 to Q4 on the basis of DII with Q1=Most anti-inflammatory and Q4= Most inflammatory diet.

Figure 5.6: Distribution of study participants based on their Dietary Inflammatory Index (DII) score.



5.6.2- Baseline characteristics of study participants categorized by Dietary Inflammatory Index (DII) quartiles.

Table- 5.3: Baseline characteristics (Mean±SD) across the quartiles of the DII.

Parameter	Q1 (N=82)	Q2 (N=76)	Q3 (N=77)	Q4 (N=75)	P value
Age(yr)	53.8 ± 10.7	55 ± 11	54.1 ± 11.5	57.6 ± 10.1	0.1
Total household income(INR)	22873±42674	17662±10683	14986±5901	17123±13176	0.24
BMI	29.3 ± 2.7	28.9 ± 2.5	29.0 ± 2.7	28.6 ± 2.8	0.51
Waist circumference(cm)	88.3 ± 6.6	88.6 ± 7.2	88.7 ± 7.2	88.9 ± 7.3	0.98
Neck circumference(cm)	33.5 ± 2.4	33.7 ± 2.5	33.5 ± 2.4	34 ± 2.5	0.58
Resting heart rate	76.9 ± 11.8	76.2 ± 13.1	77.6 ± 13.1	75.6 ± 12	0.83
Pulse-pressure difference	100.41±14.36	97.57± 15.86	95.71± 17.94	97.95± 12.47	0.84
Total cholesterol	221.5±38.4	224.3±39.8	223.5±41.6	222.9±38.4	0.97
Triglycerides	354.67±116.9	329.1±124.9	348.2±113.2	346.1±117.9	0.61
High Density Lipoprotein	52.8± 10	53.5 ± 9.6	54.0 ±10.5	54.5 ± 10.2	0.69
Low Density Lipoprotein	183.72±35.85	175.82±35.34	179.43±38.29	177.95±38.29	0.63
Fasting Blood Glucose	129.87±50.87	157.86±49.29	157.53±50.27	148.18±52.79	0.006
HbA1C	6.11±1.77	7.13±1.72	7.12±1.75	6.77±1.85	0.004
METS(physical activity)	127.44±93.42	122.75±84.78	118.61±98.59	98.61±91.57	0.21

Table 5.3 Analysis of baseline parameters across these DII quartiles revealed no statistically significant differences indicating a relatively balanced distribution of these characteristics across the spectrum of dietary inflammatory potential in the study cohort.

Conversely, significant differences were observed for both Fasting Blood Glucose (FBG) and HbA1c across the DII quartiles. FBG levels varied significantly ($p=0.006$), with a mean of 129.87 ± 50.87 mg% in Q1 (most anti-inflammatory) and progressively higher means in Q2 157.86 ± 49.29 mg%, Q3 157.53 ± 50.27 mg%, and Q4 148.18 ± 52.79 mg%. Similarly, HbA1c levels showed a significant difference ($p=0.004$), with Q1 exhibiting a mean of 6.11 ± 1.77 and higher means in Q2 (7.13 ± 1.72), Q3 (7.12 ± 1.75), and Q4 (6.77 ± 1.85). These results suggest an association between dietary inflammatory potential and glycemic control parameters in the study population.

Figure 5.7: The mean levels of fasting blood glucose levels across the DII quartiles.

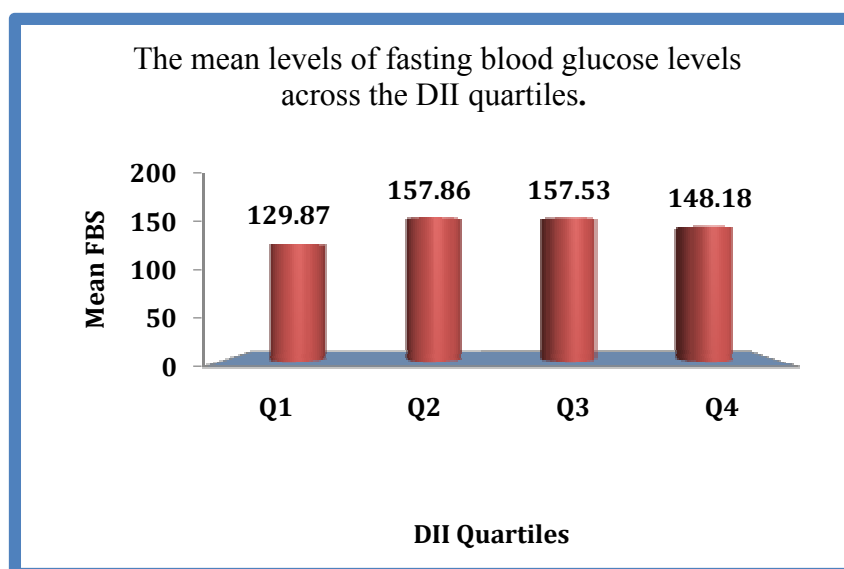
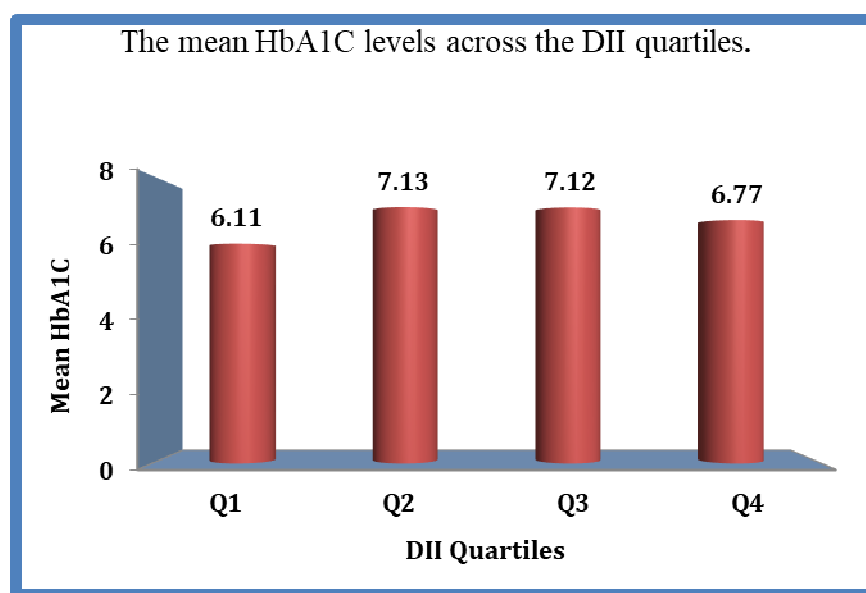


Figure 5.8: The mean HbA1C levels across the DII quartiles.



It is noted that the mean FBS levels and HbA1C levels rise as the inflammatory potential of the diet increases (Q1 to Q3), though the mean FBS levels tend to decline in the fourth quartile relative to the third quartile, it still tends to be higher than seen in the first quartile and the observation aligns with the complex interactions between dietary inflammation, glycemic control, and cardiovascular disease (CAD) risk.

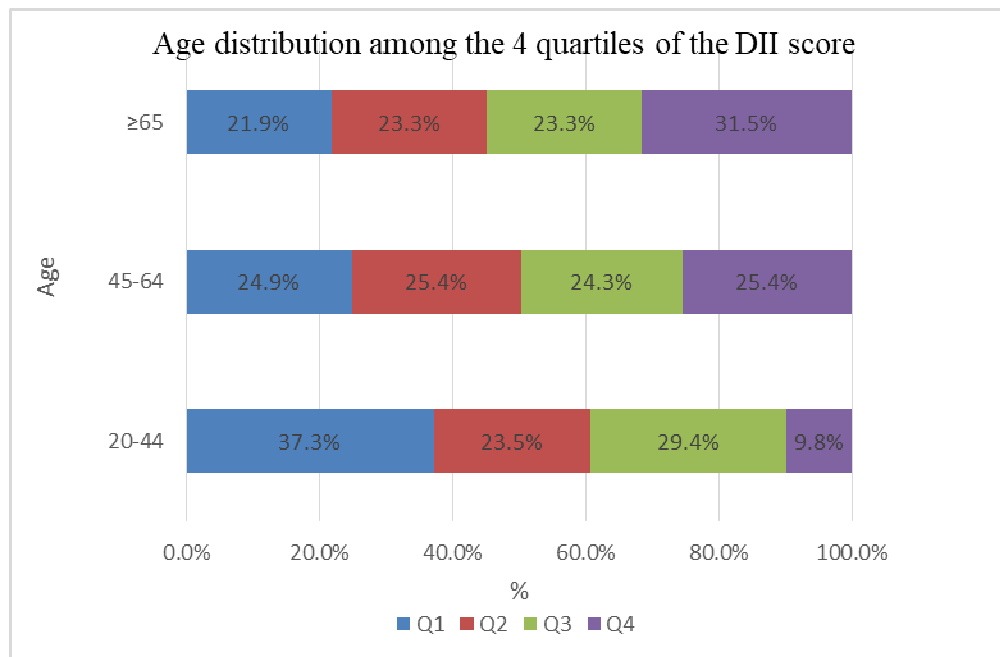
5.6.3- Association between baseline demographic, anthropometric, clinical, and biochemical characteristics and Dietary Inflammatory Index (DII) quartiles.

In our study we aimed to determine the association between the baseline characteristics and the quartiles of the Dietary Inflammatory Index (DII), using chi-square test of independence for categorical variables and Cochran-Armitage trend test to detect trends in proportions across ordered groups.

Table- 5.4: Baseline characteristics and their association with DII Quartiles.

Parameter	Q1 (N=82)	Q2 (N=76)	Q3 (N=77)	Q4 (N=75)	P value
Age(yr)					
20-44	20(24.4%)	12(15.8%)	16(20.8%)	6(8%)	p=0.01
45-64	46(56.1%)	47(61.84%)	44(57.1%)	46(61.3%)	
≥65	16(19.5%)	17(22.3%)	17(22.1%)	23(30.7%)	
Sex					
Male	56(68.3%)	47(61.84%)	50(64.9%)	53(70.7%)	p=0.68
Female	26(31.71%)	29(38.1%)	27(35%)	22(29.3%)	
Diet					
Vegetarian	60(73.2%)	65(85.5%)	59(76.6%)	63(84%)	p=0.32
Non-vegetarian	22(26.8%)	11(14.5%)	19(24.7%)	12(16%)	
Education					
Primary	28(24.4%)	22(29%)	22(28.6%)	25(33.3%)	p=0.23
Secondary	19(23.2%)	21(27.6%)	19(24.7%)	22(29.3%)	
Graduation	35(42.7%)	33(43.4%)	36(46.8%)	28(37.3%)	
BMI					
Normal	2(2.4%)	3(3.95%)	9(11.7%)	7(9.3%)	p=0.12
Over weight	43(52.4%)	39(51.3%)	39(50.7%)	39(52%)	
Obese	37(45.1%)	34(44.7%)	29(37.7%)	29(38.7%)	
Waist circumference(cm)					
Low risk	41(50%)	31(40.8%)	39(50.7%)	33(44%)	p=0.88
High risk	27(32.9%)	34(44.7%)	23(29.9%)	29(38.7%)	
Very high risk	14(17.0%)	11(14.5%)	15(19.5%)	13(17.3%)	
HTN	29(35.4%)	41 (53.9%)	45(58.44%)	44(58.7%)	p=0.01
Dyslipidemia	67(81.2%)	57(75%)	63(81.9%)	62(82.7%)	p=0.73
DM	32(39.0%)	40(52.6%)	41(53.2%)	56(74.6%)	p=0.03
Tobacco	18(21.95%)	19(25%)	19(24.7%)	18(24%)	p=0.88
Smoking	40(48.8%)	43(56.6%)	42(54.6%)	35(46.7%)	p=0.72

Figure 5.9: Age distribution among the 4 quartiles of the DII score.

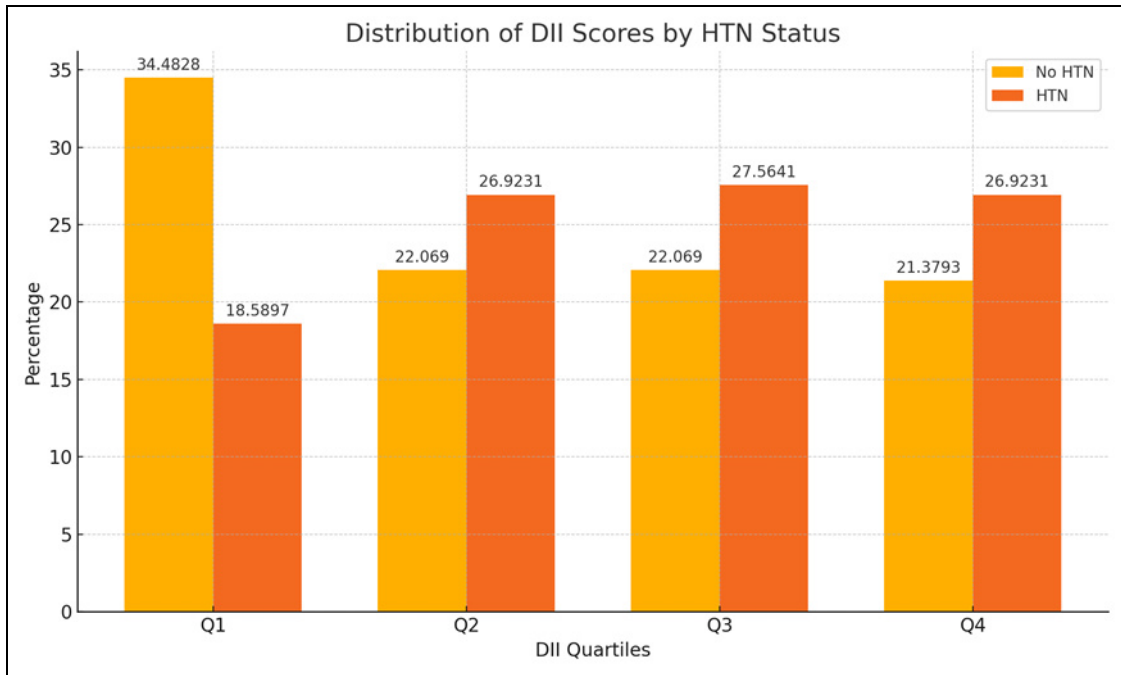


Bivariate analysis was performed using the Cochran-Armitage trend test to examine associations between age and DII quartiles, which had a p value=0.01. There is a statistically significant trend in the proportions of DII scores across the age groups. Specifically, the proportion of CAD patients in the higher age group (≥ 65) in the highest DII quartile (Q4) are more (N=23) while the proportion of CAD patients in the same age group in the lowest quartile (Q1) are less (N=16). This suggests that older age groups tend to have higher DII scores, indicating a potential age-related increase in dietary inflammation.

Table- 5.5: Association of DII Quartiles with different age groups.

Age	DII							
	Q1		Q2		Q3		Q4	
	Count	%	Count	%	Count	%	Count	%
20-44	19	37.3%	12	23.5%	15	29.4%	5	9.8%
45-64	44	24.9%	45	25.4%	43	24.3%	45	25.4%
≥65	16	21.9%	17	23.3%	17	23.3%	23	31.5%
Total	79	26.2%	74	24.6%	75	24.9%	73	24.3%
p=0.01								

Figure 5.10: Status of hypertension across the 4 quartiles of the DII score.

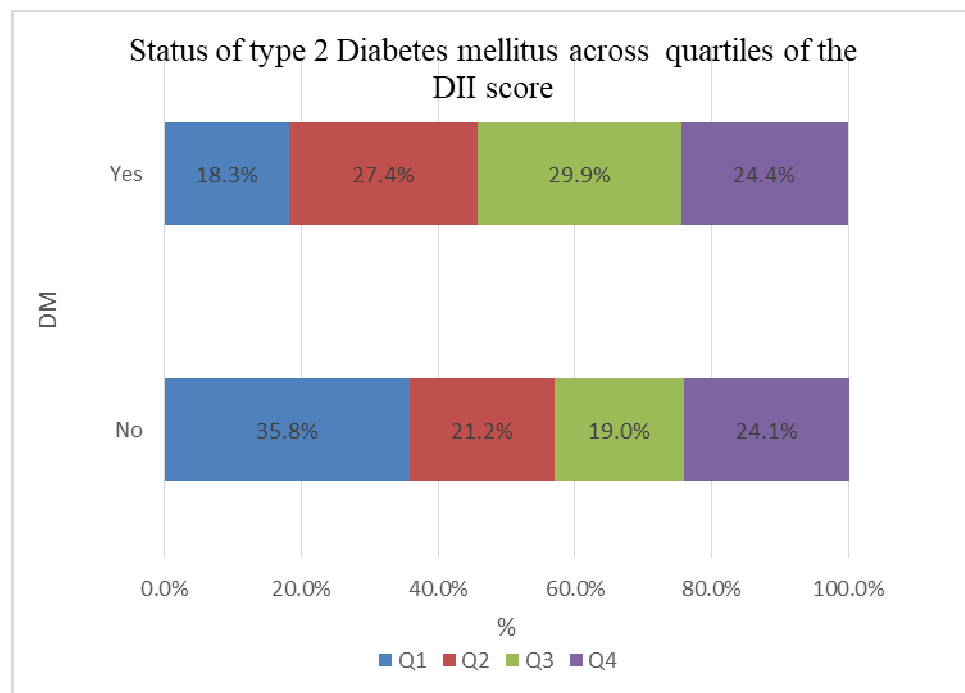


There is a statistically significant trend in the proportions of DII scores across hypertension status. Specifically, the proportion of CAD patients without hypertension is higher (N=53) in the lowest DII quartile (Q1) and lower (N=33) in the highest DII quartile (Q4), while the opposite i.e, CAD patients with hypertension (N=42) are more often categorized as Q4 as compared to Q1 with individuals having hypertension(N=29). This suggests that CAD patients with hypertension tend to have higher DII scores, indicating a potential association between higher dietary inflammation and the presence of hypertension.

Table- 5.6: Association of DII Quartiles with Hypertension status.

HTN	DII							
	Q1		Q2		Q3		Q4	
	Count	%	Count	%	Count	%	Count	%
Present	29	18.6%	42	26.9%	43	27.6%	42	26.9%
Total	82	26.4%	76	24.6%	77	24.9%	75	24.3%
p=0.01								

Figure 5.11: Association of type 2 Diabetes mellitus (DM) status and quartiles of the DII.



A significant trend ($p=0.03$) is observed in the proportions of DII scores across the condition of DM. Specifically, the proportion of CAD patients with DM ($N=30$) is lower in the Q1 DII and higher in the Q4 DII with CAD patients with DM ($N=40$). This shows that the inflammatory potential of the diet could play a contributory role in pathogenesis of the DM.

Table- 5.7: Association of DII Quartiles with DM status.

DM	DII							
	Q1		Q2		Q3		Q4	
	Count	%	Count	%	Count	%	Count	%
Present	30	18.3%	45	27.4%	49	29.9%	40	24.4%
Total	82	26.2%	76	24.6%	77	24.9%	75	24.3%
p=0.03								

5.7- Serum inflammatory markers and Dietary Inflammatory Index (DII) quartiles.

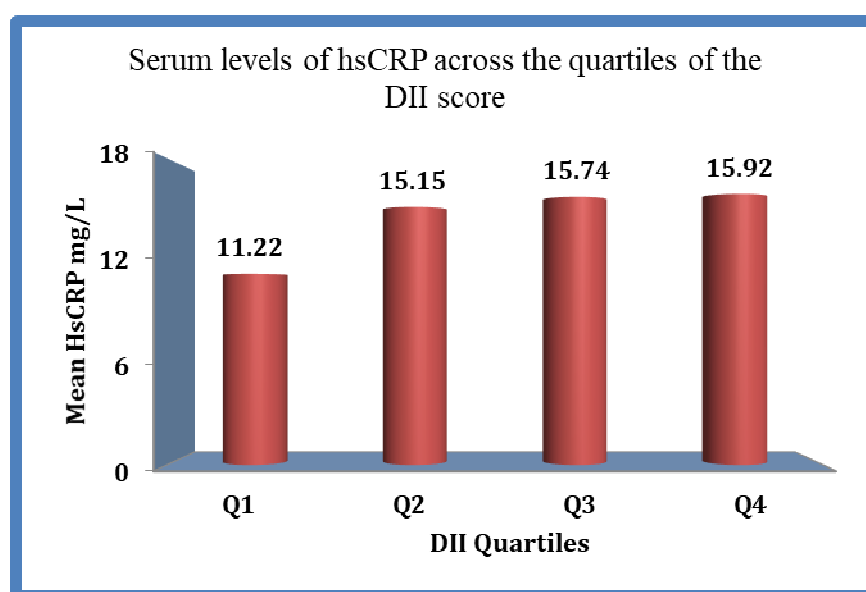
To know whether there are any statistically significant differences between the mean levels of serum inflammatory markers from Q1 to Q4 One-Way ANOVA was applied.

Table- 5.8: Association of serum inflammatory markers and the quartiles of the DII.

Parameter	Q1 (N=82)	Q2 (N=76)	Q3 (N=77)	Q4 (N=75)	P value
HsCRP mg/L	11.22± 7.19	15.15± 7.3	15.74± 7	15.92± 7.98	<0.001
TNF alpha pg/ml	172.33±46.63	222.55±55.73	113.87±40.02	121.09±48	0.09
IL-10 conc. pg/ml	165.23±65.27	76.20± 28.32	58.47± 20.65	32.4±17.17	<0.001

5.7.1-HsCRP levels: A statistically significant difference in HsCRP levels is noted across the four quarters (Q1 to Q4) and the hsCRP levels show a consistent rise from Q1 to Q4. The above findings suggest that a potential trend of increasing inflammatory load is seen across the DII quartiles and the DII score effectively indicates possible contribution of the dietary pattern to the systemic inflammation as indicated by serum levels of HsCRP.

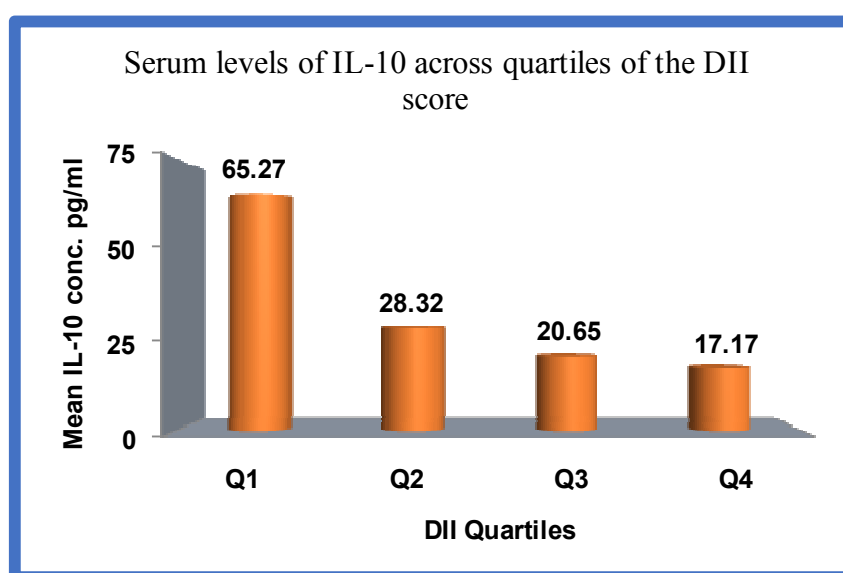
Figure 5.12: Serum levels of hsCRP across the quartiles of the DII score.



5.7.2-TNF alpha levels: Though, there is considerable variability in TNF alpha levels observed across the quartiles in the present study, highest mean levels are observed in Q2 while Q3 has the lowest. Literature suggests that low TNF alpha levels alone are not sufficient to predict outcomes and understanding how TNF alpha levels modulate systemic inflammation is crucial.

5.7.3-IL-10 levels: The mean IL-10 levels decrease significantly from Q1 to Q4, and also the decrease is statistically significant. Low levels of IL-10 might suggest a failure to regulate the immune response properly, potentially leading to unchecked inflammation. It could also indicate disease progression where the body is less able to control inflammation.

Figure 5.13: Serum levels of IL-10 across the quartiles of the DII score.



5.8-DII score and the severity of the CAD disease

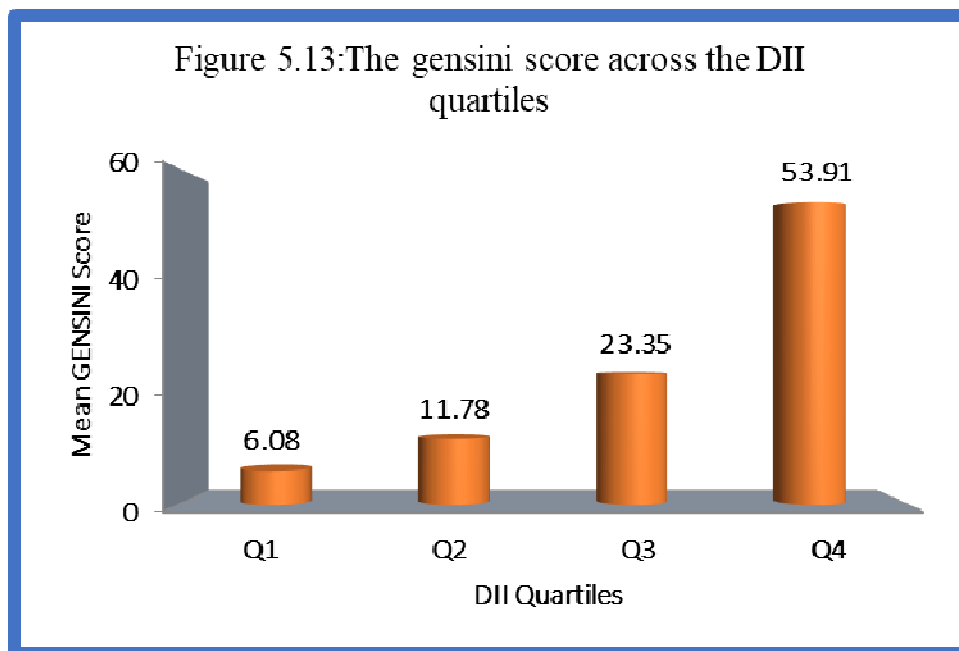
The Gensini score provides a comprehensive method to quantify the severity of coronary artery disease, taking into account both the degree of stenosis and the importance of the lesion's location within the coronary artery tree. A higher Gensini score indicates more severe coronary artery disease. The Gensini score helps in stratifying patients based on the severity of their coronary artery disease, which can guide treatment decisions and risk management and it is also useful for Prognosis where a higher Gensini scores are associated with worse clinical outcomes, including higher risks of myocardial infarction and cardiovascular mortality. Furthermore, the score is often used in clinical research to correlate the severity of coronary artery disease with other clinical parameters and outcomes.

In our study, we recorded the gensini score of all the study participants by their cardiac angiograms. The gensini score ranged from 0 to 174, with a mean SD of 23.38 ± 26.00 . The increase in the Gensini score from Q1 to Q4 is statistically significant which signifies that the severity of the coronary artery disease increases significantly with the increase in the dietary inflammation which is reflected by a higher DII score.

Table- 5.9: The Gensini score across the DII quartiles

Variable	DII Quartiles	Range	Mean $\pm$ SD	p-value
GENSINI Score	Q1	0.0 - 82.5	6.08 $\pm$ 11.14	<0.001
	Q2	0.0 - 53	11.78 $\pm$ 9.98	
	Q3	0.0 - 60	23.35 $\pm$ 13.17	
	Q4	0.0-174	53.91 $\pm$ 31.47	

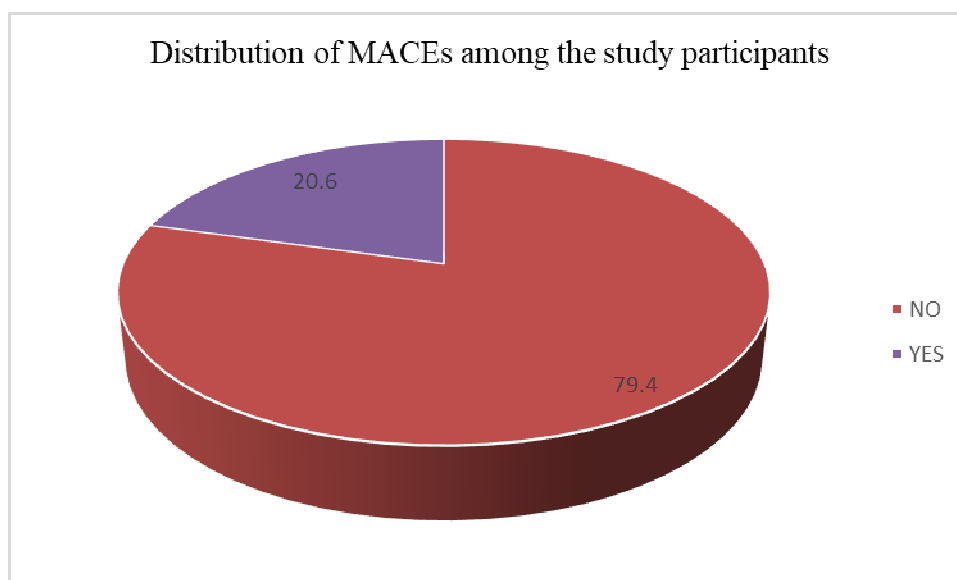
Figure 5.14: Gensini score across the 4 quartiles of the DII score.



5.9 -DII score and the major adverse cardiac events (MACEs)

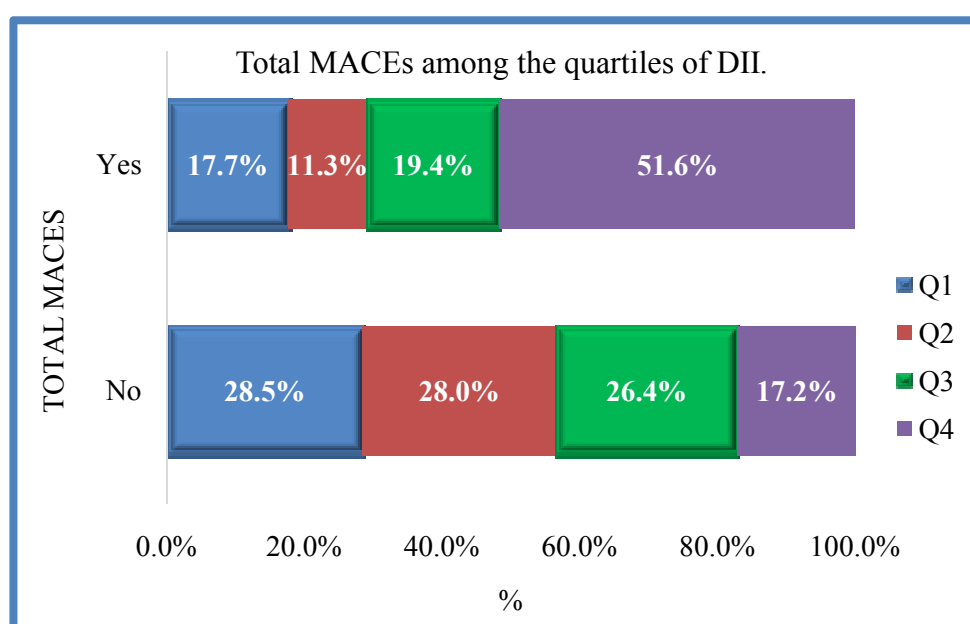
In our study, the CAD patients were followed for a month to document the major adverse cardiac events (MACEs) in them. They were recorded for each of the study participants.

Figure5.15: Distribution of MACEs among the study participants.



About 20.6% of the study population suffered major adverse cardiac events on a follow up period of 30 days after the first diagnosis.

Figure 5.16: Total MACEs among the quartiles of DII.



5.10- Various Major adverse cardiac events (MACEs) among the study cohort

Table 5.10: Distribution of the MACEs among the study participants.

Parameter	N (Frequency %)
OPD	154(51.2%)
Emergency requiring hospitalizations	23(7.6%)
Heart Failure	3(1.0%)
Cardiac arrest (Resuscitated)	26(8.6%)
Cardiac Procedure	42(14.0%)
Death	6(2.0%)

Among the 310 study participants who were followed for a thirty day period after the first diagnosis, forty eight (15.6%) were without any significant complaints warranting any visit to the consulting physician/cardiologist while 159 (51.2%) had to visit for OPD services for minor complaints. About 33.22 % of the total CAD patients under study had various MACEs recorded and about 2% of the CAD patients died within 30 days of the first diagnosis and care.

Figure 5.17: Death as a Major Adverse Cardiac Event among the study participants.

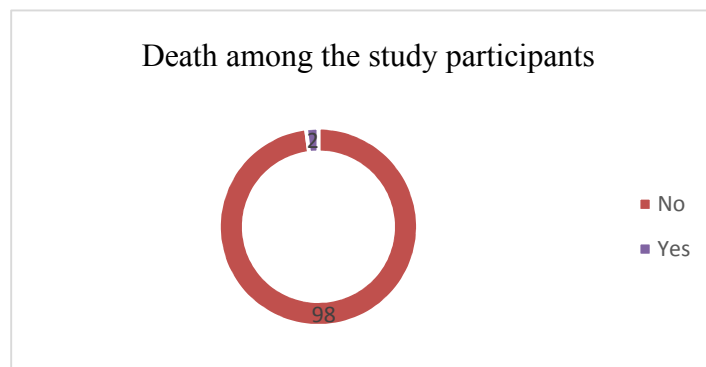


Table 5.11: Association of the various MACEs among the DII quartiles.

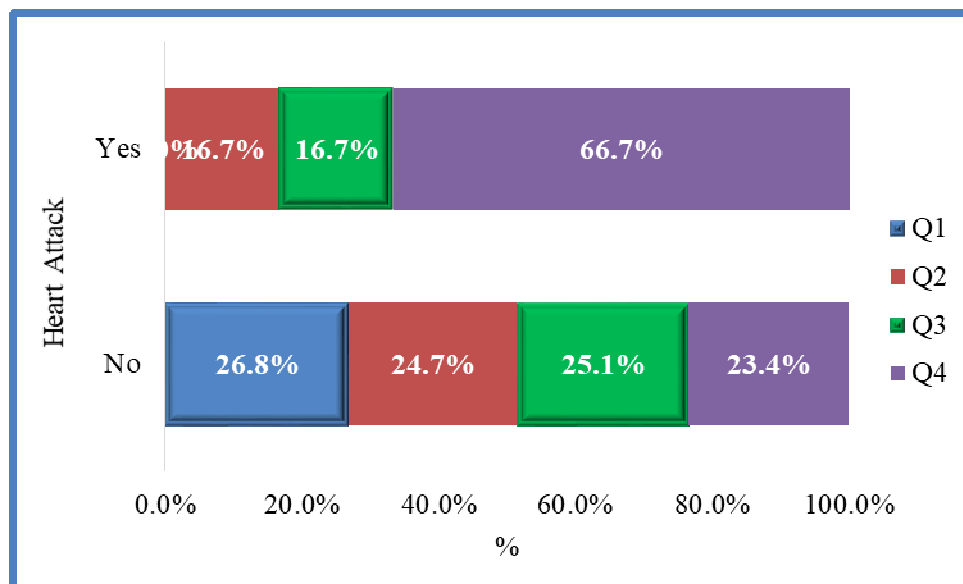
30day follow-up	Q1 82(26.2%)	Q2 76(24.6%)	Q3 77(24.9%)	Q4 75(24.3%)	P value
Emergency requiring hospitalisations	9 (39.2%)	4 (17.4%)	5 (21.7%)	5 (21.7%)	p=0.35
Myocardial infarction	0 (0%)	1(16.7%)	1(16.7%)	4(66.7%)	p=0.02
Ischaemic Stroke	0(0%)	2(33.3%)	1(16.7%)	3(50.0%)	p=0.13
Heart Failure	0 (0%)	0 (0%)	1(33.3%)	2(66.7%)	p=0.06
Cardiac arrest (Resuscitated)	5(19.2%)	6(23.1%)	8(30.8%)	7(26.9%)	p=0.49
Cardiac procedure-urgent revascularisation	6(14.3%)	2(4.8%)	3(7.1%)	31(73.8%)	p<0.001
Death	0(0%)	1(16.7%)	1(16.7%)	4(66.7%)	p=0.02
Total MACEs	11(17.7%)	7(11.3%)	12(19.4%)	32(51.6%)	p<0.001

When the MACEs were tabulated for the DII quartiles, we observe that the frequency (%) of total MACEs were higher in Q4 (51.6%) than in Q1 (17.7%) and was statistically significant with p value <0.001.

Furthermore, the data suggest a significant association between higher DII quartiles (indicating more pro-inflammatory diet) and increased incidence of second myocardial infarction among CAD patients (p=0.02).

5.10.1- Second MI as a Major adverse cardiac events (MACEs) among the study cohort

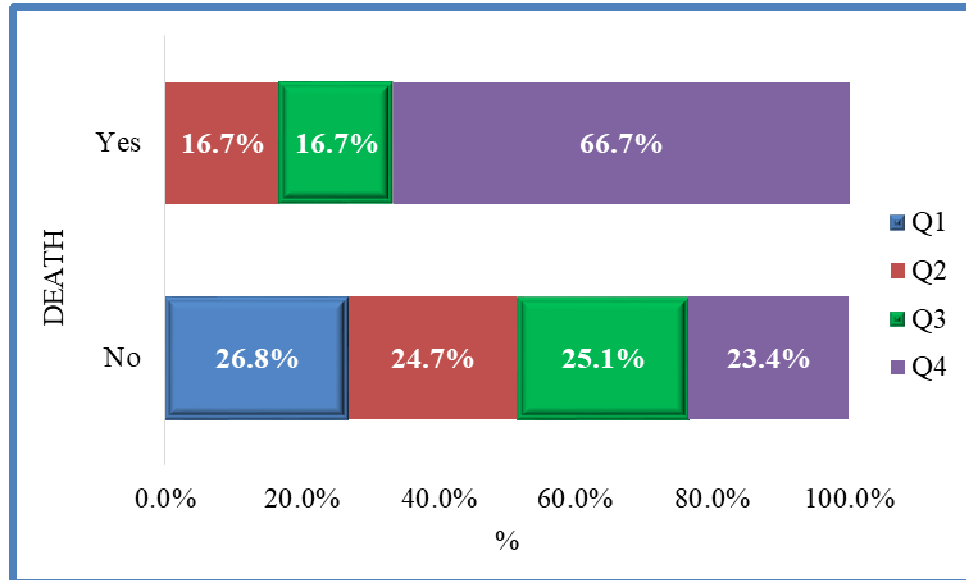
Figure 5.18: Distribution of second MI among the quartiles of DII.



Although not statistically significant ($p=0.06$), there is a suggestive trend towards higher rates of heart failure as DII scores increase, particularly notable in Q4. The rates of cardiac arrest that required resuscitation show variability across DII quartiles indicating that DII scores do not significantly influence the occurrence of resuscitated cardiac arrests among CAD patients. A significant variation in the frequency of cardiac procedures across DII quartiles. Notably, patients in Q4 (highest DII scores) undergo significantly more cardiac procedures ($p<0.001$) compared to those in lower quartiles, suggesting a potential association between higher DII scores and more frequent need for cardiac interventions.

5.10.2- Death as a Major adverse cardiac events (MACEs) among the DII quartiles.

Figure 5.19: Death as one of the MACEs among the quartiles of DII.



It is also noteworthy that mortality rates differ significantly across DII quartiles and patients in Q4 have a significantly higher rate of death ($p=0.02$) compared to other quartiles, indicating that higher DII scores may be associated with increased mortality among CAD patients.

These findings suggest that higher DII scores, indicative of a more pro-inflammatory diet, may correlate with worse outcomes such as increased mortality, second MI and need for cardiac procedures among patients with CAD. Further research could explore mechanisms linking dietary inflammation to cardiovascular outcomes to better inform dietary interventions in CAD management.

5.11- DII as a predictor of MACEs in the study cohort

In our study, Multivariate analysis involved a forward stepwise logistic regression model to investigate the association between DII quartiles and the MACEs.

Table 5.12: Association of the MACEs with the DII quartiles. (Unadjusted odds ratios)

DII	Exp(B)	p-value	95% C.I.for EXP(B)	
			Lower	Upper
Q1	Ref			
Q2	0.65	0.39	0.24	1.77
Q3	1.18	0.72	0.48	2.86
Q4	4.82	<0.001	2.20	10.60

The odds of experiencing MACEs are significantly higher in Q4 compared to Q1 (Reference), with an odds ratio of 4.82. This difference is statistically highly significant ($p < 0.001$), suggesting a robust association between higher DII scores (Q4) and increased risk of MACEs. The results of the forward stepwise logistic regression model indicate that CAD patients with higher DII scores (Q4) are about 5 times an increased risk of MACEs among patients with Coronary Artery Disease (CAD). These findings suggest that higher DII scores, may independently contribute to adverse cardiovascular events in CAD patients.

We do recognize that the risk of MACEs is not determined by a single factor, but rather by the complex interplay of several factors. Adjusting for demographic, anthropometric, lifestyle and co-morbid conditions is crucial for investigating the association between Dietary Inflammatory Index (DII) and Major Adverse Cardiovascular Events (MACEs)

Demographic factors (such as age, gender), anthropometric measures (BMI, waist circumference), lifestyle factors (smoking, physical activity), and co-morbid conditions (diabetes, hypertension) are all potential confounding variables and these variables can independently influence both DII scores and the occurrence of MACEs. Failing to account for them could lead to biased estimates of the association between DII and MACEs.

Hence, we calculated adjusted odds ratio for investigating the association of the MACEs and the DII.

Table 5.13: Association of the MACEs with the DII quartiles. (Adjusted odds ratio)

DII	Exp(B)	p-value	95% C.I. for EXP(B)	
			Lower	Upper
Q1	Ref			
Q2	1.41	0.382	0.78	1.88
Q3	2.37	0.672	0.92	1.97
Q4	2.52	0.060	2.5	4.76

The association of the MACEs with the DII quartiles becomes insignificant after adjusting for Demographical, Anthropometric, Lifestyle and Co-morbid conditions. CAD patients whose diet shows the most proinflammatory nature with a highest DII score among the studied population (Q4) shows a strong potential increase in the risk of MACEs, though statistically insignificant. This suggests that individuals in the highest DII quartile (Q4) may be at substantially greater risk of MACEs, though the p-value indicates this should be interpreted with caution.

CHAPTER 6

DISCUSSION

The current study brings forth valuable insights into the relationship between inflammatory potential of the diet, systemic inflammation, and adverse outcomes in patients with coronary artery disease (CAD) consuming a traditional North Karnataka diet.

The study cohort consisted of more males (67%) than females (33%) with a mean age of 55 ± 10 years (median age=56 year). The most prevalent risk factor observed was dyslipidemia, affecting a large majority of the cohort (82.7%, n=256), followed by diabetes mellitus (54.5%, n=169), smoking (53.2%, n=165), hypertension (51.8%, n=159) and also a substantial proportion of the cohort were noted to be overweight.

In our study, The DII score ranged between -2.01 to +6.38 with a mean $\pm$ SD of 2.28 ± 1.75 indicating a predominantly pro-inflammatory dietary pattern within the cohort. We grouped the study participants on the basis of DII score into quartiles - Q1 to Q4 (Quartile 1: -2.01 to +1.24; Quartile 2: +1.25 to 2.34; Quartile 3: 2.35 to 3.24; and Quartile 4: 3.25 to 6.38) for the convenience of statistical application. Q1 representing the most anti-inflammatory and Q4 as the most inflammatory diet taken by the study cohort.

6.1- Comparison of demographic characteristics among the DII quartiles

When the study cohort were grouped on the basis of DII score into quartiles, most of the demographic (gender, dietary preference, tobacco, smoking and education), anthropometric (BMI, waist circumference) and biochemical parameters (dyslipidemia) were evenly distributed, indicating a relatively uniform distribution across the spectrum of dietary inflammatory potential. However, statistically significant difference were noted in the status of hypertension ($p=0.01$), DM ($p=0.03$) and it was also noted that the Q4 had a larger proportion of CAD patients above the age of 65 year ($p=0.01$).

The Q1, Q2, Q3, and Q4 quartiles had about 35.4%, 53.9%, 58.44% and 58.7% of hypertensive patients respectively among the CAD cohort. A growing body of evidence in the recent past highlights the crucial role of chronic low-grade inflammation in the pathogenesis and progression of HTN. This can be in cumulation to the well-established traditional risk factors such as genetics, obesity, and lifestyle

choices. Similar findings were noted by numerous researchers like Zeng F et.al, and Zhao H et.al, where a direct and significant association between higher DII scores and an increased risk as well as prevalence of hypertension was observed which remained significant even after adjusting for confounding factors (1).

Similar observations were also made across different age groups from adults to adolescents and among various populations. (2,3,4). Furthermore, a recent meta-analysis has observed a dose-response relationship between DII score and the incidence of HTN, with a (relative risk = 1.04; 95% CI: 1.00-1.07) it further discerns that for each one-point rise in the DII score, the incidence of hypertension rose by four percent which is substantial in clinical practice. [2]

A positive linear correlation between higher DII scores and an increased likelihood of youth hypertension was also noted among Americans [4].

These observations further extended in hypertensives and the Higher DII scores have also been associated with increased use of antihypertensive medications [3] which further establishes the notion that a more inflammatory diet progressively escalates the risk of developing high blood pressure.

Dietary inflammation can contribute to the development of HTN by accelerating many underlying pathogenic mechanisms like endothelial dysfunction which leads to an impairment in the production of vasodilators like nitric oxide which leads to vasoconstriction and increased peripheral resistance characteristic of hypertension [5, 6].

High DII also causes an increase in the oxidative stress further reducing the bioavailability of nitric oxide, and vascular remodeling, all of which contribute to elevated blood pressure [7,8]. An incessant chronic inflammation contributed by dietary patterns can also possibly activate the Renin-Angiotensin-Aldosterone System (RAAS) directly or indirectly and cause elevated blood pressure.

RAAS activation causes an increased renin release from the kidneys which leads to a rise in the production of angiotensin II and also aldosterone which by the virtue of being a potent vasoconstrictor cause increased sodium and water retention leading to HTN.re [9].

Hypertension is also endowed by vascular stiffness and renal sodium retention caused by Immune Cell activation and infiltration leading to the release of additional pro-inflammatory mediators which perpetuate the inflammatory cycle. [10, 11).

Furthermore, pro-inflammatory diets are also often linked to increased adipose Tissue inflammation releasing adipokines and cytokines that contribute to systemic inflammation, insulin resistance, and activation of the sympathetic nervous system, all of which are linked to hypertension [12].

These evidences further supports the concept that dietary components have an potential impact on the systemic inflammation, possibly act as active modulators of inflammatory pathways that directly impact vascular health and blood pressure regulation.

Our study also reported a statistically significant association between the high DII scores and the condition of type2 DM in CAD patients. A similar positive relationship is also corroborated by numerous studies and the association was also found to be extended to hyperglycemia, hyperinsulinemia, and insulin resistance (prediabetes.) (Measured by HOMA-IR) [13,14]

Even the risk of developing T2DM was significantly higher for the individuals in the highest quintile of DII scores when compared to those in the lowest quintile as reported by the researchers from Iran(15) and a separate study from Mexico(16) further added that the odds of developing T2DM were even stronger in older adults. Conversely, anti-inflammatory dietary patterns tend to significantly reduce FPG and HbA1c levels in T2DM patients [17].

These findings are annotated by the fact that dietary inflammation (high DII) leads to an upsurge of IL-1 β , TNF- α , and IL-6 which are proinflammatory cytokines.

These cytokines can impair insulin secretion and cause β -cell apoptosis [17, 18] and contribute to β -cell dysfunction and eventually to insulin resistance which are central to the development and progression of T2DM [17, 10].

Our findings further strengthen the recent study findings which consistently demonstrate that pro-inflammatory diet indicating inflammation remains one of the crucial factors in the nuanced relationship between DM and CAD. This connection is not merely coincidental but is mechanistically driven by chronic systemic inflammation, a fundamental pathological process common to both conditions.

6.2- Dietary inflammation and inflammatory markers

6.2.1- Association between DII and hsCRP

Our study reported a potential trend of increasing serum levels of hsCRP across the Q1 to Q4 of the DII quartiles (Table 5.8) indicating a rising inflammatory load. This points to a significant link between dietary choices and the circulating levels of hsCRP which is a widely recognized and a robust biomarker of systemic inflammation.

Similar findings were reported by a study on Korean adults (19) which noted that the individuals who were grouped in the highest quintile of DII scores had an increased risk (70%) of raised levels of hsCRP. Furthermore, a recent meta-analysis also documented that higher DII scores had a 21% increased risk for elevated CRP levels (20). Apart from reflecting the systemic inflammation, hsCRP can prove to be of significant prognostic value in assessing the risk and progression of CAD.

It is now evidenced that the risk of myocardial infarction, stroke, and sudden cardiac death which constitute the major cardiovascular events can also be predicted by raised hsCRP levels alone and this is true for even the apparently healthy population and also in those with well-controlled traditional risk factors [21,22] it is also now recognized that higher hsCRP levels correlate with the progression of arterial plaque formation in the heart and the CAD severity. [22].

The diet through its components which are pro-inflammatory in nature like for e.g., saturated and trans fats, refined carbohydrates, excessive use of red and processed meats can be the triggers that activate immune responses [20, 23] that lead to a rise in the circulating cytokines including hsCRP and IL-6. Oxidized lipoproteins, advanced glycation end products (AGEs), and imbalances in gut microbiota can also act as similar triggers in the individuals partaking inflammatory diets. [21,24].

6.2.2- Association between DII and TNF- α

TNF alpha is a key pro-inflammatory cytokine mainly functioning in the regulation of immune cells, and its persistent production can lead to prolonged inflammation and tissue damage. Our study noted that there is considerable variability in TNF alpha levels across the quartiles with Q2 of the CAD cohort having the highest mean levels and the lowest ranges are observed in Q3.(Table 5.8)

Augmented levels of serum TNF- α has a crucial role in compounding the process of atherosclerosis, advancing plaque instability and in turn increase the occurrence of MACEs in CAD patients. Many studies have also observed a positive correlation between higher DII scores and raised TNF- α levels in them.(25) CAD severity as assessed by scoring systems like the SYNTAX score or Gensini score show a significant correlation with increased TNF- α levels (26)

The noted correlation between the DII score and TNF- α levels are not only true in CAD patients but also in healthy or apparently healthy populations, along with other markers like HsCRP) and IL-6 which reflect an inflammatory state. This low grade inflammation chronic in nature seen even before the overt manifestation of the disease itself is touted to be a significant risk factor for the development of chronic diseases.(27)

The correlation between the DII score and the TNF- α levels is not always noted to be linear but is modulated by other factors, obesity being an important one. Obese individuals often exhibit higher DII scores as the visceral adipose tissue is known to secrete many of the pro-inflammatory adipokines, including TNF- α . This increases the systemic inflammation in them manifold suggesting a synergistic effect of a pro-inflammatory diet and obesity. (7)

In the context of Coronary Artery Disease (CAD), which is fundamentally an inflammatory disease, Tumor Necrosis Factor- α (TNF- α) is a major pro-inflammatory cytokine that significantly contributes to various underlying pathological processes that drive the development and progression of atherosclerosis, including endothelial dysfunction, Increased Adhesion Molecule Expression, Smooth Muscle Cell Proliferation and Migration ultimately leading to atherosclerosis progression, plaque instability, and myocardial injury (28) . Plaque instability and subsequent rupture in acute coronary syndromes is critical and is a consequence of raised TNF- α causing increased degradation of the extracellular matrix within the plaque.(29)

This is evidenced by studies in animal models which have documented that genetic deletion or inhibition of TNF- α offers protection against atherosclerotic lesion formation. (30)

But the findings in our study do not conform to the above observations. This can be explained by the fact that the expression of TNF- α is thought to be a highly complex biological process which is driven by myriad of genetic, environmental, and lifestyle factors. This may lead to the variations in the levels observed across individuals having different DII scores.

Genetic predispositions of individuals may respond to the same pro-inflammatory dietary stimulus recorded as similar DII scores but lead to differential TNF- α responses. For eg.

Single nucleotide polymorphisms (SNPs) in the TNF- α gene promoter region, can significantly influence its transcriptional activity and, consequently, protein expression levels leading to more robust TNF- α production. (31)

Apart from the genetic variations in TNF- α among individuals, it is known to be produced by various cell types like macrophages, T cells, and endothelial cells and also has variable expression levels and distribution of TNF- α receptors (TNFR1 and TNFR2) on target cells. Different DII scores may influence the activation state of these different cell populations and the subsequent receptor expression, leading to diverse inflammatory responses even with comparable circulating TNF- α levels. (32)

In summary, though a distinct relation is evident between the pro-inflammatory diets (higher DII) and increased TNF- α levels, precise role and intensity of TNF- α expression seem to be modulated by a complex interplay of genetic factors, cellular responses, the inflammatory architecture and the metabolic programming in the individual. This complex regulation makes it tough to accurately predict the TNF- α response using just DII scores, emphasizing the need for personalized diets that tend to tame the systemic inflammation and help manage inflammatory conditions such as CAD.

6.2.3- Association between DII and IL-10

Our study reveals a significant inverse relationship between the IL-10 levels and DII scores, where the mean IL-10 levels decrease significantly from Q1 (165.23 ± 65.27) to Q4 (32.4 ± 17.17). This finding is critical as it suggests a direct dietary influence of the principal components in elevating the inflammatory milieu by not only raising the pro-inflammatory markers but also downgrading the body's anti-inflammatory defenses.

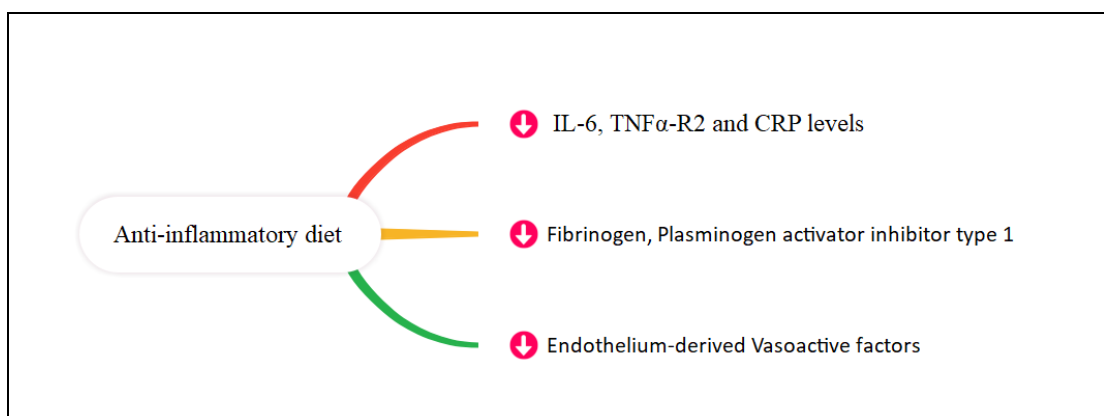
Given that the DII was developed and validated to predict inflammatory biomarker concentrations, including IL-10, it's expected that higher DII scores would lead to reduced IL-10 levels. [33].

Despite numerous studies theorizing that pro-inflammatory diets (indicated by a high DII) should suppress IL-10—a key anti-inflammatory cytokine—these studies have primarily shown only an association between high DII and elevated levels of pro-inflammatory markers like hs-CRP and IL-6, rather than directly demonstrating IL-10 suppression. (34-36)

However, some studies like the one conducted on the north Indian population found that

serum IL-10 levels were significantly lower in patients with CAD when compared to healthy controls. They also observed higher levels of TNF- α and a significantly increased TNF- α /IL-10 ratio in CAD patients which are similar to our findings and significant too. (37)

Figure 5. 20 : Anti-inflammatory diet and Inflammatory markers.



Another study that was done in the patients with metabolic syndrome noted that a lower incidence of severe CAD among them were associated with higher IL-10 levels. These findings supported the direct protective role of IL-10 when its levels are robust enough to counteract inflammation effectively [38] This further suggests that personalized nutrition that can help maintain adequate IL-10 through an anti-inflammatory diet could be crucial in preventing the *initiation* or *progression* of CAD, before the inflammatory cascade becomes overwhelming. The discussion on the association of IL-levels and DII scores in CAD patients is incomplete without our attention to certain specific study findings that have been documented.

Few studies (39-40) have recorded a paradoxical association of elevated IL-10 with increased adverse outcomes in established CAD and with that of increased CHD risk. These observations, though may seem counterintuitive, they do not disprove IL-10's role in being anti-inflammatory in nature. Infact these observations might suggest that in patients with severe or chronic CAD, a possible up regulation of IL-10 is necessitated by a persistently high inflammation and the elevated levels of IL-10 may be a compensatory drive by the body s immune system.

However, the efforts to neutralize an overwhelming pro-inflammatory load can be limited and thus are exposed as an association of high IL-10 with advanced disease. Thus, High IL-10 levels observed among CAD patients with advance disease or with severe complications draws the attention to the complexities and the dynamic interplay of cytokines in chronic inflammatory diseases. [41].

In addition to the various known mechanisms like those involving the direct activation of inflammatory pathways, Some of the dietary components in High DII dietary pattern create an environment conducive to chronic systemic inflammation by stimulating toll-like receptors (TLRs) on immune cells, leading to the activation of nuclear factor kappa-B (NF-κB), a master regulator of inflammatory gene expression, ultimately boosting the production of pro-inflammatory cytokines and potentially suppressing anti-inflammatory ones like IL-10 [41].

Some of the dietary components in High DII dietary pattern can stimulate toll-like receptors (TLRs) on immune cells, leading to nuclear factor kappa-B (NF-κB) activation, a master regulator of inflammatory gene expression causing a rise pro-inflammatory cytokines synthesis and potentially suppressing anti-inflammatory ones like IL-10 [42].

The role of other known mechanisms like oxidative stress (43), and gut microbiome dysbiosis (44) and even the direct activation of the pro-inflammatory cytokines by the pro-inflammatory diets cannot be disregarded.

6.3 Dietary inflammation and CAD severity (GENSINI score)

Our findings reveal a significant direct positive correlation between the higher DII scores and CAD severity, based on the Gensini scoring system ($p < 0.001$). Specifically, patients diagnosed with CAD who exhibited higher DII scores (Q4) were found to have commensurately higher Gensini scores (53.91 ± 31.47) when compared to those with lower DII(Q1) who had lower CAD disease (6.08 ± 11.14). This observation underscores the potential role of dietary inflammatory potential in influencing the progression and severity of CAD.

Commonly when the CAD has to be assessed for its extent and severity to decide for the further course of management the Gensini score is a widely accepted and robust quantitative angiographic scoring system. The gensini system assigns numerical values based on the degree of stenosis and location of coronary artery lesions, with higher scores indicating more severe and extensive atherosclerosis. Hence providing an objective and a precise measure for evaluating the burden of CAD. (45) The ability of the gensini score to capture the extent and functional significance of coronary lesions, as highlighted in studies on its predictive value for clinical outcomes, reinforces its utility in demonstrating the impact of dietary inflammation. (46)

Gensini scoring system was hence chosen for a more nuanced understanding of its relationship with the inflammatory potential of the dietary pattern. While some research studies observed that diabetic patients had significantly higher Gensini scores which reflect a more severe and extensive CAD. These observations implicitly highlight the role of metabolic inflammation in CAD progression, a concept that can be linked to DII. Our current study extends this understanding by showing dietary inflammation, as quantified by DII, corresponds to this objective measure of disease burden. (47)

Our study findings are not an isolated observation but rather adds to a growing body of evidence confirming this association across diverse populations

and methodologies. This consistency strengthens the understanding that pro-inflammatory dietary patterns contribute significantly to the severity of coronary artery disease (CAD). Similar research conducted in diverse populations, such as the study by Dadaei et al. in Iranian adults, also reported a robust association between higher DII scores and greater angiographic severity of CAD, as assessed by the Gensini score. This consistency across different ethnic and geographical contexts strengthens the generalizability of the link between dietary inflammatory potential and atherosclerotic burden. (13)

NHANES analysis, which is one of the large population-based studies illustrated an independent association between DII and overall CHD risk which hugely substantiate the concept underlying our findings that a pro-inflammatory diet may not only promote vascular inflammation directly but also exert their detrimental effects on CAD severity through various metabolic dysregulations contributing to plaque formation and progression, ultimately leading to higher Gensini scores. They also mentioned other indices that help assess the risk objectively like triglyceride-glucose index, visceral adiposity index.(48)

Some studies employed other measures of atherosclerotic burden like increased carotid intima-media thickness (CIMT) (49) or coronary artery calcium (CAC) scores (50) both of which are established markers of subclinical atherosclerosis and its severity. The results published are consistent with our findings which further solidifies the link between dietary inflammation and disease progression.

The consistent demonstration of a positive correlation between dietary inflammatory potential and the severity of coronary artery disease, as observed in our study and others, strongly indicates that dietary patterns are a significant and modifiable determinant of atherosclerotic progression. This emphasizes the clinical imperative for dietary interventions to mitigate inflammatory processes, which can in turn contribute to reducing the burden of CAD and potentially influencing Gensini scores.(51)

6.4 Dietary inflammation and MACEs in CAD Patients: A Critical Link

Major Adverse Cardiac Events (MACEs) often serve as strong predictors or direct components of CAD mortality. (52)

Our study recorded MACEs for each of the study participants. Of the total CAD patients under study, 33.22 % reported various Major Adverse Cardiac Events and the recorded mortality was at about 2%.The present study was conducted with the primary objective to inquire if the MACEs were proportionally higher in those with higher dietary inflammation (read as high DII score) which is reflected as higher levels systemic inflammation and increased severity of the disease (gensini score) which are evidenced to be the forbearance of the MACEs.

We found that the CAD patients in the Q4 (high DII) had higher total incident MACEs (51.6%) when compared to Q1 (low DII) who had 17.7 %.(Table 5.9). These are significant as existing literature (53) consistently links higher DII scores to increased cardiovascular disease (CVD) risk factors, incidence, and mortality. Research on metabolic syndrome reports a link between higher DII scores elevated all-cause and cardiovascular disease-related mortality, implying a greater risk of MACE in this vulnerable population. (54)

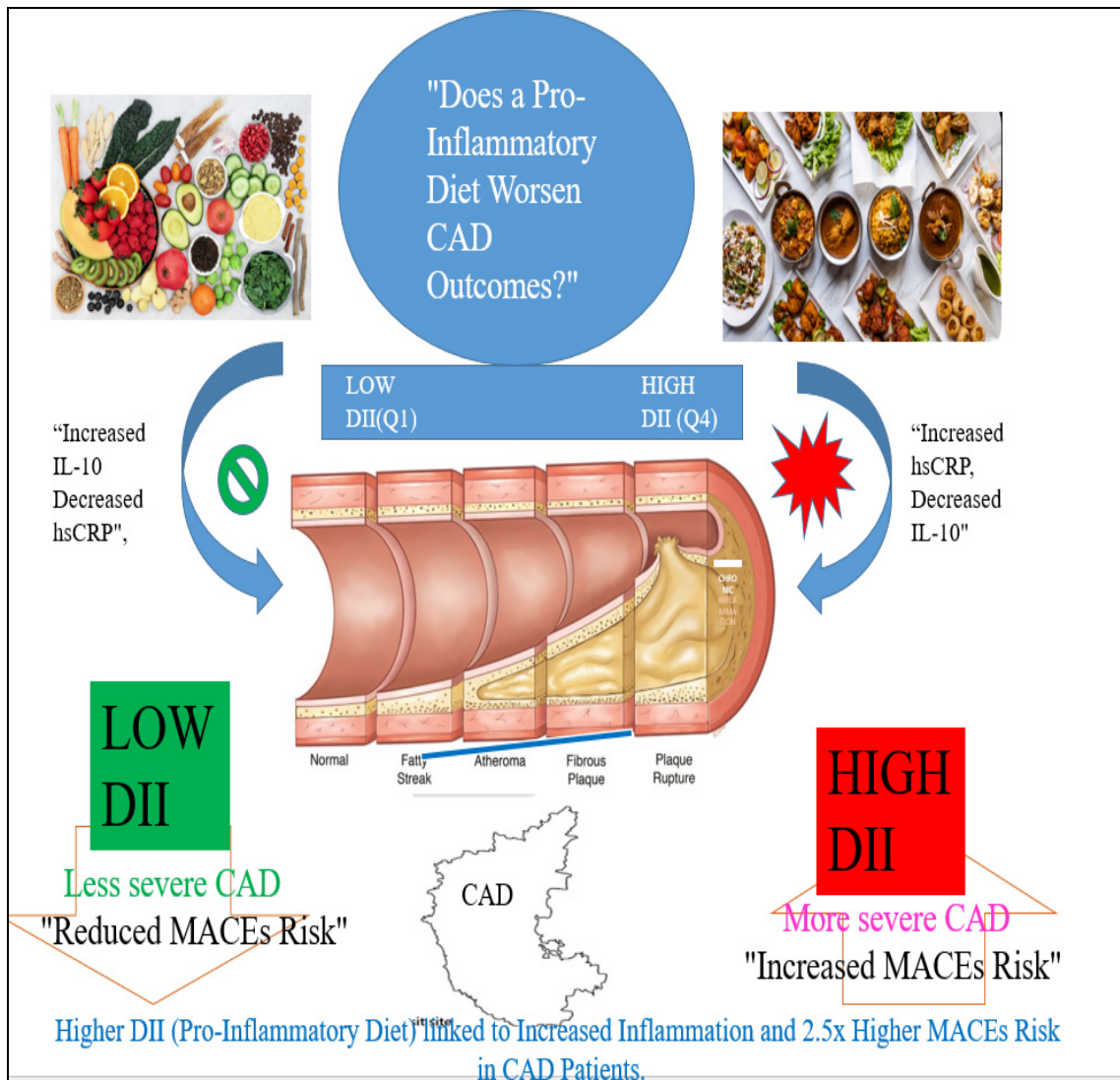
However, explicit studies directly correlating DII with MACEs in populations with established Coronary Artery Disease (CAD) remain notably limited. The influence of dietary inflammation on MACE is not limited to primary prevention but also impacts individuals with pre-existing conditions. Our study addresses this critical gap by robustly demonstrating an association between higher DII scores in CAD patients and a greater incidence of various MACEs. Higher DII scores, indicative of a more pro-inflammatory diet correlate with worse outcomes such as increased mortality, second MI and need for cardiac procedures among patients with CAD. These findings provide a crucial link in the continuum of disease progression, suggesting that dietary inflammatory potential contributes not only to the risk of developing CAD and the severity of underlying atherosclerosis but also to the ultimate occurrence of MACEs and subsequent mortality which further underscores the clinical relevance of our observations.The research findings and their inference are underpinned by the established role of chronic low-grade inflammation in

atherothrombosis. A pro-inflammatory diet, as captured by higher DII scores, promotes systemic inflammation, leading to endothelial dysfunction, oxidative stress, and the activation of pro-atherogenic pathways. These processes lead to more extensive and diffuse form of Coronary Artery Disease, destabilize atherosclerotic plaques, increasing the likelihood of rupture and subsequent thrombotic events, which are the direct cause of myocardial infarction and ischemic stroke, central to MACE.(55,56)

Bridging the Gap – DII, CAD Severity, and MACEs

Thus the robust correlation between high DII of the north Karnataka population and known risk factors (HTN, DM and old age), Inflammatory-Anti-inflammatory Equilibrium (elevated hsCRP and decreased IL-10) along with increased severity of the disease (gensini score) and ultimately the occurrence of MACEs in CAD patients becomes significant and can offer a pivotal contribution, serving to both advance the scientific understanding of disease mechanisms and to directly inform and enhance clinical care strategies.

Figure 5.21: Graphical presentation of the study



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CHAPTER 7

SUMMARY

Summary and conclusion

DII score of the study population ranged from -2.01 and +6.38 with a mean DII score of 2.28 ± 1.75 and ~89% of the study participants had pro-inflammatory dietary pattern (DII >0.0); A statistically significant increase in serum levels of HsCRP (proinflammatory) and a significant decrease in the IL-10 levels (anti-inflammatory) were noted in Q4 (high DII score) which indicates increased chronic subacute systemic inflammation in them. The odds of experiencing MACEs are significantly higher in Q4 compared to Q1 (Reference), with an odds ratio of 4.82. This difference is highly statistically significant ($p < 0.001$), suggesting a strong association between higher DII scores (Q4) and increased risk of MACEs, particularly second myocardial infarction ($p=0.02$) and the need of cardiac procedures ($p<0.001$) though this association becomes insignificant after adjusting for the known confounding variables. CAD patients with higher DII score had increased mortality ($p=0.02$) when compared to those with lower DII score.

Research Hypothesis: “The major adverse cardiac events in CAD patients occur more often in those with higher DII scores and is reflected as higher inflammatory load has been proved.

Clinical implications

Most of the current pharmaceutical measures aimed to reduce the chronic systemic inflammation are not without complications. Thus, identification of anti-inflammatory agents with favorable safety records has always been a priority. Modifying diet- inclusion of more anti-inflammatory nutrients in the context of a behavioral or lifestyle intervention among individuals with overt CAD could prove to be more safe and an effective supplementary therapeutic tool for the specific population under study. The diet with lower DII score has the potential to reduce the onset or the progression of existing disease resulting in reduced mortality, morbidity burden and healthcare cost for the individual and the households or the country at large.

Research implications

The study findings shall further help us derive a more robust dietary risk assessment and modification, one which is evidenced based and acceptable with ease by the locals.

It will also add immense value in our efforts to develop dietary interventional strategies in preventing cardiac events at large.

It can also be a useful tool for promoting clinical and public health by conducting counseling and educational activities on health and nutrition.

Limitations of the study

1. Possibility of response bias in diet data collection cannot be entirely ruled out. However, the Food Frequency Questionnaire (FFQ) used was validated to reflect local dietary patterns and was administered by trained interviewers. Any exposure misclassification is expected to be ‘non-differential’, which would likely underestimate the association between DII and CAD risk factors and the occurrence of MACEs.
2. Second, the cross-sectional nature of the analysis prevents establishing temporality (direction of causation) between dietary inflammation, inflammatory markers and MACEs among CAD patients. Longitudinal studies are needed to determine the temporal sequence of these associations.
3. Third, is the Short follow up period- The 30 day follow up period, while providing valuable information, is a relatively short period of time. A longer follow up period would have been beneficial.

Future perspective

1. Need extensive research- such as intervention studies to confirm the causality of the associations between diet, inflammation and MACEs in Coronary artery disease patients or research in different populations.
2. It is recommended people that in order to reduce the inflammatory potential of the diet, people in this region should minimize the consumption of foods which are calorie dense and nutrient-poor / fried foods. Consciously, they should also increase the consumption of nutrients dense foods high in antioxidants, vitamins and minerals.

ANNEXURES



BLDE (DEEMED TO BE UNIVERSITY)

Annexure -I

PLAGIARISM VERIFICATION CERTIFICATE

1. Name of the Student: **Dr. Deepa S Sajjanar** Reg No: 15PHD001
2. Title of the Thesis: "Dietary inflammatory index of north Karnataka food pattern and its association with coronary artery diseases".
3. Department: Biochemistry
4. Name of the Guide & Designation: **Dr. Indira A Hundekari**
Professor,
SBMPMC, BLDE (DU), Vijayapura.
5. Name of the Co-Guide & Designation: **Dr. Rekha Udigiri**
Professor of Community Medicine and **Dr V G Warad**, Professor
of Medicine, SBMPMC, BLDE (DU), Vijayapura.
6. The above thesis was verified for similarity detection. The report is as follows:

Software used: iThenticate Date: 20-06-2025

Six Percent
Similarity Index (06 %): Total word Count: 19,381

The report is attached for the review by the Student and Guide. The plagiarism report of the above thesis has been reviewed by the undersigned. The similarity index is below accepted norms.

The similarity index is above accepted norms, because of following reasons:

.....
.....The thesis may be considered for submission to the University. The software report is attached.

Signature of Guide	Signature of Coguide	Signature of Student	Verified By (Signature)
Name & Designation	Name & Designation	Name & Designation	Name & Designation

Shree 20/6/25
Librarian
B.L.D.E. Deemed to be University
Shri B. M. Patil Medical College,
Vijayapur.

Proforma

Dietary inflammatory index of north Karnataka food pattern and its association with Coronary Artery Diseases.

1. Demographic data

Name: Age: Sex: Religion:

2. Socioeconomic data

Highest level of education: Total household income:
Employment status: Marital status: PIN code:

3. Vital statistics:

Height: Weight: Waist circumference: Heart rate:

Blood pressure (sitting position; average of 3 measures):

4. Pertinent medical history

Hypertension : Hyperlipidemia: Myocardial infarction:
Percutaneous coronary intervention: Coronary artery bypasses graft:
Congestive heart failure requiring treatment: Diabetes mellitus:
Peripheral artery disease (known $\geq 50\%$ or moderate or severe stenosis; history of revascularization):
Carotid artery disease (known $\geq 50\%$ or moderate or severe stenosis; or history of revascularization):
Cerebrovascular disease (clinical history of TIA or stroke):
Abdominal aneurysm:
Chronic renal dysfunction (creatinine > 1.5 mg/dL):

Inflammatory condition:

Malignancy:

5. Smoking history

Use of >100 cigarettes /other forms of tobacco in lifetime:

Use within 6 months (current vs former):

6. Baseline medications (for at least the 7 days prior visit)

7. Left ventricular ejection fraction

8. Indication for coronary angiography

9. Number of coronary arteries diseased ($\geq 50\%$ diameter stenosis in an artery with ≥ 2 mm caliber; or physiologically significant by fractional flow reserve; or previously revascularized)

Gensini score :

syntax score:

10. Blood parameters

Fasting blood glucose (FBG):

Blood urea nitrogen (BUN):

Serum albumin:

Serum creatinine:

Serum hs-CRP:

IL-10:

TNF- α :

Hb A₁C:

Hematocrit: (HCT)

other investigation details if any:

Interim events (or at the end of thirty days)

Emergency room visit with or without observation or inpatient admission

Heart attack (details of cardiac biomarkers collected if answered affirmatively)

Stroke or mini-stroke

Hospitalization for heart failure

Resuscitated cardiac arrest

Cardiac procedure (including coronary angiography and/or coronary revascularization)

Outpatient clinic visits

Cardiac rehabilitation visits

Death:

PARTICIPANT INFORMATION SHEET AND CONSENT FORM

Title of study: Dietary inflammatory index of north Karnataka food pattern and its association with Coronary Artery Diseases.

Name of investigator and institution: Dr. Deepa S Sajjanar (Ph D student), Department of Biochemistry, BLDEU's Shri B.M. Patil Medical College, Hospital & Research Centre, Vijayapur- 586 103.

Name of sponsor: None

Introduction: You are invited to participate in a research study because you have coronary artery disease and the disease is known to have Major adverse cardiac events such as non-fatal MI, non-fatal stroke, and CVD-related death which could possibly have an association with the diet pattern that can increase the inflammatory load and cause the above consequence.

It is important that you understand why the research is being done and what it will involve.

Please take your time to read through and consider this information carefully before you decide if you are willing to participate. Ask the study staff if anything is unclear or if you'd like more information. After you are properly satisfied that you understand this study, and that you wish to participate, you must sign this informed consent form. To participate in this study, you may be required to provide your doctor with information on your health history and you may harm yourself if you are not truthful with the information provided. Your participation in this study is voluntary. You do not have to be in this study if you do not want to. You may also refuse to answer any questions you do not want to answer. If you volunteer to be in this study, you may withdraw from it at any time. If you withdraw, any data collected from you up to your withdrawal will still be used for the study. Your refusal to participate or withdrawal will not

affect any medical or health benefits to which you are otherwise entitled. This study has been approved by the institutional Ethics Committee of BLDEU's Shri B.M.Patil Medical College, Hospital & Research Centre, Vijayapur.

What is the purpose of the study?

The purpose of this study is to assess whether the major adverse cardiac events in CAD patients occur more often in those with higher DII scores and are reflected as higher inflammatory load. A total of 60 subjects like you in the hospital will be participating in this study. The whole study will last about one year and your participation will be about 40 days in total.

What will happen if I decide to take part and What kind of study products will I receive?

If you agree to participate in the study, the doctor may need to perform some tests and examination to determine if you are suitable for the studies which are already a part of your treatment protocol and you will be followed for a period of 30 days and administered a FFQ to capture the Dietary inflammatory scores of the Karnataka food pattern that you have been consuming for a major part of your life. You will not be receiving any other products apart from your treatment protocol.

Study Activities Table

	Screening and enrollment	Baseline Assessment	Treatment	Follow up
Visit	1	1	1	2
Timeline (wks)	1	1	1	5
Procedures				
Informed consent	•			
Check eligibility	•			
FFQ administered	•			
Blood sample for screening	•			
Subject demographics	•			

Medical History	•			
Complete physical exam	•			
Height (cm),	•			
Weight (kg)	•			
Vital signs (Blood Pressure, pulse rate, Weight, Temperature)	•			•
Biochemical parameters(Fasting blood glucose, serum lipids, albumin, blood urea nitrogen (BUN), creatinine, hemoglobin (Hb) A ₁ C and hematocrit (HCT) hs-CRP, and TNF- α as inflammatory biomarkers and IL-10	•			
Electrocardiogram	•			
Echocardiogram	•			
Follow up				•

Screening visit and baseline (Visit 1)

Before any study-related tests and procedures are performed, you will be asked to read and sign this consent document. You will have 1 screening visit to determine if you qualify to take part in this study. You will be asked questions on your medical history and have a complete clinical examination. You will have some blood sampled, approximately 5-10 ml (2 teaspoon). The tests and procedures noted in the study activities table will be performed. If based on the screening tests and procedures, you qualify to participate in this study; the study doctor will contact or schedule you to attend further tasks related to the research study. Baseline assessment

will be carried out which shall involve recording Subject demographics and complete medical History and a thorough Complete physical exam which includes noting the Height (cm), Weight (kg) Vital signs (Blood Pressure, pulse rate, Weight, Temperature) Electrocardiogram and Echocardiogram assessment.

A FFQ being administered to capture the dietary intake which will be interviewer based and shall be used to calculate the DII of the diet pattern. A Blood sample for estimation of Biochemical parameters like Fasting blood glucose, serum lipids, albumin, blood urea nitrogen (BUN), creatinine, hemoglobin (Hb) A₁C and hematocrit (HCT) ~~hs~~-CRP, and TNF- α as inflammatory biomarkers and IL-10 shall be drawn.

Follow up

This is the final visit is 4-5 weeks after the end of your treatment visit. You will be interviewed for any re admissions to a hospital and assessed for any major adverse cardiac events. You will be given a plan from your doctor for continuation of care after the end of this study. If there are any abnormalities detected in your blood investigation, your study doctor will contact you as soon as possible.

What are my responsibilities when taking part in this study?

It is important that you answer all of the questions asked by the study staff honestly and completely. If your condition or circumstances change during the study, you must tell the study doctor. There may be certain medications (anti inflammatory)~~that~~ you cannot take while participating in this study. The doctor will discuss those medications with you. You must not take any other medications without consulting your study doctor. You must inform your study doctor immediately if you make any changes to any of your current treatments, even those which you have been taking for a long time. It is very important that your study doctor be informed

very rapidly of any eventual changes to your health during your participation in the study. For your own security, it is important that you follow your study doctor's instructions throughout the entire duration of the study.

What kind of treatment will I receive after my participation in the trial?

You will continue on standard treatment for treating your coronary artery disease and the condition you are at the present as deemed appropriate by your consultant. Whether you complete the study or withdraw early, your doctor will discuss the best alternatives for your future treatment with you.

What are the potential risks and side effects of being in this study?

Side effects from blood sampling Pain, bruising, bleeding or other discomfort at the blood drawing site have been seen and explained to you in detail.

Costs You will not be charged for the study drug or procedures during the study apart from your standard protocol treatment. The costs of hospital visits during the study will remain the same.

Please ask your study doctor if you need more information on risks and side effects. The trial staff will inform you in a timely manner about any new findings or changes about the study procedure which may affect your health or willingness to continue in this study. Where necessary, you may be asked to re consent to participate.

What are the benefits of being in this study?

There may or may not be any benefits to you. You will not be reimbursed or paid for participation in this study. However, information obtained from this study will help improve the

treatment or management of other patients with the same disease or condition. Your condition may get better, it may get worse or it may stay the same.

What if I am injured during this study?

If you are injured as a result of being in this study, you should contact your study doctor. In the event of a bodily injury or illness directly resulting from the study product or a medical procedure required for this study which is deemed nil as it is just an observational study and the calculated risk is just above minimal. You will not be paid whatsoever. The investigator is not responsible for medical expenses due to pre-existing medical conditions, any underlying diseases, any ongoing treatment process, your negligence or willful misconduct, the negligence or willful misconduct of your study doctor or the study site or any third parties. You do not lose any of your legal rights to seek compensation by signing this form.

What are my alternatives if I do not participate in this study?

You do not have to participate in this study, you can state at any stage of the study and shall be obliged as it is not an interventional study, no alternate treatment is available.

Who is funding the research?

No agency is sponsoring the study and is purely an observational and self funded study.

Can the research or my participation be terminated early?

The study doctor or the sponsor may due to concerns for your safety, stop the study or your participation at any time. If the study is stopped early for any reason you will be informed and arrangements made for your future care. You may be asked to attend a final follow-up visit

Will my medical information be kept private?

All your information obtained in this study will be kept and handled in a confidential manner, in accordance with applicable laws and/or regulations. When publishing or presenting the study results, your identity will not be revealed without your expressed consent. Individuals conducting this study and involved in your medical care, qualified monitors and auditors and governmental or regulatory authorities have access to your medical records and data and may inspect and copy your medical records, where appropriate and necessary. Your biospecimens may be sent to laboratories for testing. If this is required, your biospecimens will be coded and information that can identify you will be removed. Only your study doctor and study staff will be able to link the code with you. Data from the study will be archived. With your permission your family doctor will be informed of your participation in the study. You will be informed of the study findings.

Who should I call if I have questions?

If you have any questions about the study or if you think you have a study related injury and you want information about course of your treatment, please contact the study doctor at site:

BLDEU's Shri B.M.Patil Medical College, Hospital & Research Centre Vijayapur- 586 103,
Dr. Deepa S Sajjanar (Ph D student), Department of Biochemistry, mobile no: 8277656601

If you have any questions about your rights as a participant in this study, please contact: The Secretary, institutional Ethics Committee, BLDEU's Shri B.M.Patil Medical College, Hospital & Research Centre Vijayapur- 586 103.

B. L. D. E. U'S SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE,
BIJAPUR

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT : Dietary inflammatory index of north Karnataka food pattern and its
association with Coronary Artery Diseases.

PRINCIPAL INVESTIGATOR : Dr. Deepa S Sajjanar
Ph D student

GUIDE'S NAME : Dr J.G. Ambekar
Professor
Department of Biochemistry

CO GUIDE'S NAME : Dr. V G. Warad
Professor
Department of Internal medicine

CO GUIDE'S NAME : Dr. REKHA. UDGIRI
Professor
Department of Community medicine

1: PURPOSE OF RESEARCH: I have been informed that this study will help to profile the dietary inflammatory index of Coronary Artery Disease patients with primarily north Karnataka food pattern and assess the association between DII, serum levels of inflammatory markers and MACES, it will also help to establish diagnostic discriminatory cut off values for CAD in this population of north Karnataka for hs CRP, TNF – alpha and IL-10. This study will be useful academically as well as clinically.

2: PROCEDURE: I understand that, the procedure of the study will involve withdrawal of 5ml of venous blood in fasting state at the beginning of the study. It will also involve documentation of the various physical and test parameters requested as a part of my disease management. The procedure will not adversely affect the outcome of my condition.

3: RISK AND DISCOMFORTS: I understand that the blood will be collected by venipuncture which will involve minor discomfort and pain.

4: BENEFITS: I understand that, my participation in the study may/may not have a direct benefit to me but the results may add a new insight with a potential benefit in diet recommendations in future.

5: CONFIDENTIALITY: I understand that, medical information produced by this study will become part of institutional records and will be subject to the confidentiality and privacy regulation of the said institute. Information of a sensitive personal nature will not be a part of medical record, but will be stored in investigators research file and identified only by a code number. The code key connecting name to numbers will be kept in a separate secured location.

If the data are used for publication in the medical literature and for teaching purposes no names will be used and other identities such as photographs, audio and video tapes will be used only with my special written permission. I

understand that I may be allowed to see the photographs and the video tapes and have the audio tapes before giving this permission.

6: REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time. Concerned researcher (Dr. Deepa S Sajjanar) is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study which might influence my continued participation. If during the study or later, I wish to discuss my participation in all concerns regarding this study with a person not directly involved, I am aware that the social worker of the hospital or the investigator of this study is available to talk with me. A copy of this consent form will be given to me to keep for careful re-reading.

7: REFUSAL OR WITHDRAWAL OF PARTICIPATION: I understand that my participation is voluntary and I may refuse to participate or may withdraw my consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital. I also understand that researcher may terminate my participation in this study at any time after she/he has explained the reasons for doing so and had helped arrange for my continued care by my physician or physical therapist if this is appropriate.

8: INJURY STATEMENT: I understand that in unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, then medical treatment will be available to me, but no further compensation would be provided. I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

I have explained to _____ (Patient/Relevant guardian)

The purpose of the research, procedures required and the possible risk and benefits to the best of my ability.

Investigator/ Guide

Date

I confirm that Dr. Deepa S Sajjanar has explained to me the purpose of research, the study procedure that I will undergo, and the possible risk and discomforts as well as benefits that I may experience. Alternative to my participation in the study have also been to give my consent from. Therefore I agree to give consent to participate as a subject and this research project.

Participant / Guardian

Date:

Witness to signature

Date:

Modified from Portney L.G, Watkins M.P., in Foundation of Clinical Research, Second Edition, New Jersey, Prentice Hall Health 2000. (APPENDIX – E)

The FFQ-NK is developed and validated for use by the principal investigator and its use is subjected to copyright.

Food Frequency Questionnaire developed for North Karnataka

Name: Gender: Age: Place (Urban/rural):.....

Income of the household:..... No. of family members:

Height:.....weight:.....Waist circumference:.....Hip circumference:.....

Over the past one year, on a typical day (excluding fasting, festivals etc.), how often have you eaten [specific food item] and what was the average portion size that you consumed [prompting from models and standard portion sizes]

1. Food Frequency Questionnaire

Food group	Units	Frequency						
Starchy staples	Serving size	2-3 times per day	1 time per day	3-4 times per week	1-2 times per week	2-3 times per month	1 time per month or less	Rarely/ never
Avalakakki	100 g							
Bread	slice							
Biscuit -type	No.							
Churumary oggarani	100 g							
Churumary chuda	100 g							
Dosa	No.							
Idli	No.							
Jowar roti	No.							
Chapati(wheat)								
Madli	100 g							
Masala rice or pulav	100 g							
Noodles	100 g							
Paddu	100 g							
Puri (wheat)	No.							
Puri (maida)	No.							
Rice	100 g							
Sajji roti	No.							
Sabudana Khicdi	100 g							
Talipatti	100 g							
Uppit	100 g							
Vermicelli upma	100 g							
Vada (uddin)	No.							
Legumes And Nuts								
Almond	No.							
Bengal gram dhal-bhaji	25 gm							
Bengal gram dal holige	No.							
Black gram- Papad	No.							
Cow pea(alasandi bhaji	25 gm							

Food group	Units	Frequency						
		2-3 times per day	1 time per day	3-4 times per week	1-2 times per week	2-3 times per month	1 time per month or less	Rarely/ never
Legumes And Nuts	Serving size							
Coconut chutney	25 gm							
Cashew	No.							
Chakkali	No.							
Green gram bhaji-heasaru bele	25 gm							
Groundnut holige	No.							
Groundnut undi	No.							
Groundnut chutney	25 gm							
Jhunka (Bengal gram) curry	25 gm							
Mothbean (madaki) bhaji	25 gm							
Niger seed chutney	5 gm							
Onion Baji	No.							
Peas (batani) bhaji	25 gm							
Kharbele (toor dal) bhaji	25 gm							
Kharbele (toor dal) sambhar	1 katori							
Shev	25 gm							
Green leafy Vegetables								
Bengal gram leaves bhaji	25 gm							
Fenugreek(methi) bhaji	25 gm							
Gogu (pundi palle) bhaji	25 gm							
Kirsali	25 gm							
Rajgira(amarnath) bhaji	25 gm							
Spinach (palak) bhaji	25 gm							
Shepu(sabsige) bhaji	25 gm							
Other vegetables								
Bottle gourd bhaji	25 gm							
Beetroot bhaji	25 gm							
Bitter gourd bhaji	25 gm							
Brinjal bhaji	25 gm							
Cabbage bhaji	25 gm							
Food group	Units	Frequency						

Other vegetables	Serving size	2-3 times per day	1 time per day	3-4 times per week	1-2 times per week	2-3 times per month	1 time per month or less	Rarely/ never
Cauliflower bhaji	25 gm							
Capsicum bhaji	25 gm							
Cluster beans bhaji	25 gm							
Drumstick	25 gm							
French beans	25 gm							
Field beans bhaji	25 gm							
Kovai(toned kai) bhaji	25 gm							
Ladies finger	25 gm							
Mekki kai bhaji	25 gm							
Ridge gourd bhaji	25 gm							
Parval	25 gm							
Pumpkin	25 gm							
Pink beans	25 gm							
Potato bhaji	25 gm							
Radish (mulangi) bhaji	25 gm							
Tomato bhaji	25 gm							
Tomato sambhar	Katori							
Egg								
Boiled egg	100 g							
Egg curry	100 g							
Egg burji	100 g							
Omlette	100 g							
Flesh foods								
Chicken gravy	100 g							
Chicken kabab	100 g							
Chicken manchurian	100 g							
Chicken biryani	100 g							
Fish fry	100 g							
Fish gravy	100 g							
Mutton biryani	100 g							
Mutton curry	100 g							
Mutton sukka	100 g							
Milk and dairy products								
Milk coffee	100 ml							
Milk horlicks	100 ml							
Curds	100 ml							
Butter milk	100 ml							
Paneer as curry	100g							
Pickles								
Lime hot	5 g							
Lime sweet	5 g							
Mango hot	5 g							
Food group	Units	Frequency						

	Serving size	2-3 times per day	1 time per day	3-4 times per week	1-2 times per week	2-3 times per month	1 time per month or less	Rarely/ never
Mango sweet	5 g							
Salads/ raw food								
Cucumber	50 gm							
Carrot	50 gm							
Mixed Salad	50 gm							
Onion	50 gm							
Radish	50 gm							
Sprouts	50 gm							
Fats and oils								
Butter	5 g							
Ghee	5 g							
Other snacks								
Gobi Manchurian	100g							
Vada pav	No.							
Kachori	No.							
Pani puri	No.							
Sweets and desserts								
Godhi huggi	100 g							
Jalebi	No.							
Peda	No.							
Sheera	100 g							
Sajka	100 g							
Vermicelli payasa	100 g							
Fruits								
Apple	100 g							
Banana	100 g							
Custard apple	100 g							
Dates	100 g							
Grapes	100 g							
Guava	100 g							
Orange	100 g							
Papaya	100 g							
Pomegranate	100 g							
Pineapple	100 g							
Sapota	100 g							
Alcoholic beverages								
Beer	30 ml							
Whisky	30 ml							
Others	30 ml							

ANNEXURE II
INSTITUTIONAL ETHICAL CLEARANCE
CERTIFICATE



BLDE
(DEEMED TO BE UNIVERSITY)

Deemed as Deemed to be University u/s 3 of UGC Act, 1956

The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA
BLDE (DU)/IEC/ 413/2019-20 **27th December, 2019**

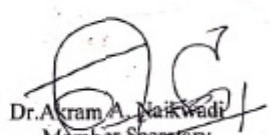
INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The ethical Committee of this University met on 27th December, 2019 at 11.00 a.m. scrutinizes the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. student College 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: *Dietary inflammatory index of north Karnataka food pattern and its association with Coronary Artery Diseases.*

Name of the Principal Investigator: Dr.Deepa Sajjanar, Asst.Professor, Dept. of Biochemistry.

Dr. Santoshkumar Jeevanagi
Chair person
IEC, BLDE (DU),
VIJAYAPURA
Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura


Dr. Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA
MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
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

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.

BLDE (DU): Phone: +918352-262770, Fax: +918352-263303, Website: www.bldeu.ac.in, E-mail: office@bldeu.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, E-mail: bmprnc.principal@bldeu.ac.in

ANNEXURE III PRESENTATIONS

1. Development and validation of a Food frequency questionnaire (FFQ-NK) for use in epidemiological studies among north Karnataka population on 6-7 August 2020, conducted by BLDE (Deemed to be university) Vijayapura.
2. Association of DII and TNF-alpha with diabetes mellitus in the CAD patients of north Karnataka on 25th and 26th April 2024. “Cracking code of life - molecular biology in action Conducted by dr prabhakar kore institute of basic sciences, Belgavi. (Awarded Certificate of Merit)
3. Inflammation and cardiovascular disease severity: How diet shapes health outcomes at PHYSICON 2024 from 15 to 17 Nov 2024, Tripura University, Agartala.



PHYSICON-2023

XXXIV Annual National Conference of The Physiological Society of India (PSI)

Theme: "Physiology: From Experimental To Translational Medicine"

Certificate

Dr. Deepa S. Sajjanar

has presented paper & won prize (PG/Ph.D/Faculty) in
PHYSICON-2023 held from 2nd & 3rd Nov 2023 Organised by
Department of Physiology, BLDE (DU) Shri.B.M.Patil Medical College,
Hospital and Research Center, Vijayapura, Karnataka (India)

G. S. S.

PRESIDENT, PSI

P. S. S.

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ORGANISING COMMITTEE

S. S.

ORGANISING SECRETARY



Dr. Prabhakar Kore Basic Science Research Center (BSRC)

KLE Academy of Higher Education & Research, (KAHER), Belagavi

Accredited A+ Grade by NAAC (3rd Cycle) & Placed in Category 'A' by MoE (GoI)



Certificate

This is to Certify that

Miss/Mr./Dr./Prof. Deepa Sajjanar has

participated in the National Conference "Cracking Code of Life - Molecular Biology in

Action' Organized by Dr. Prabhakar Kore Basic Science Research Centre, KAHER on

25th and 26th of April 2024 and presented as oral / poster presentation and
secured 3rd Prize

B. P.

Dr. Ramkishan Paranjape

Organizing Chairman, National Conference

Director, BSRC, KAHER, Belagavi, Karnataka.

Director (R&D) ICMR-National AIDS Research Institute, Pune

Dr. B.

Dr. Bhushan Kulikarni

Organizing Secretary, National Conference

Scientist Grade II, BSRC, KAHER,

Belagavi, Karnataka



XXXVth Annual Conference of The Physiological Society of India

National Seminar on

Emerging Health Issues and Challenges: Perspective from Northeast India

CERTIFICATE OF APPRECIATION

This is to certify that

Prof./Dr. Deepa S. Sajjanar.....
of
has attended the conference and seminar as Invited Speaker during November 15-17, 2024
organized by the Department of Human Physiology, Tripura University with paper entitled

Dr. Rajen Haldar
Hony. General Secretary
The Physiological Society
of India

Prof. Prasunpriya Nayak
President
The Physiological Society
of India

Prof. Dipayan Choudhary
President
Organizing Committee
PHYSICON 2024

Prof. Samir Kumar Sil
Organizing Secretary & Convenor
Organizing Committee
PHYSICON 2024

ANNEXURE IV PUBLICATIONS

1. Sajjanar DS, Hundekari IA, Udgiri R, Warad VG, Sajjannar SL. Development and Validation of a Food Frequency Questionnaire for Use in Epidemiological Studies Among North Karnataka Population. Natl J CommunityMed 2024; 15(4):289-298. DOI: 10.55489/njcm.150420243758 (SCOPUS)
2. Comparative Analysis of Dietary Inflammatory Index, Tumor Necrosis Factor-Alpha, and Metabolic Parameters in Diabetic and Non Diabetic Coronary Artery Disease Patients In North Karnataka.. Accepted December 2024 for publication.



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Development and Validation of a Food Frequency Questionnaire for Use in Epidemiological Studies Among North Karnataka Population

Deepa S Sajjanar
BLDE (DU) SBMP medical college hospital and research centre, Vijayapura, India

Indira A Hundekari
BLDE (DU) SBMP medical college hospital and research centre, Vijayapura, India

Rekha Udgiri
BLDE (DU) SBMP medical college hospital and research centre, Vijayapura, India

Vijay Warad
BLDE (DU) SBMP medical college hospital and research centre, Vijayapura, India

Sanjeev L Sajjannar
BLDE (DU) SBMP medical college hospital and research centre, Vijayapura, India

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SECTION

Original Research Articles

ABSTRACT

Background: Dietary records, such as food diaries (FD) and food frequency questionnaires (FFQs), are widely employed in large-scale epidemiological analyses. Given the regional diversity in dietary habits,

ORIGINAL RESEARCH ARTICLE

Development and Validation of a Food Frequency Questionnaire for Use in Epidemiological Studies Among North Karnataka Population

Deepa S Sajjanar^{1*}, Indira A Hundekari², Rekha Udgiri³, VG Warad⁴,
Sanjeev L Sajjanar⁵

¹⁻⁵BLDE (DU) SBMP medical college hospital and research centre, Vijayapura, India

DOI: 10.55489/njcm.150420243758

ABSTRACT

Background: Dietary records, such as food diaries (FD) and food frequency questionnaires (FFQs), are widely employed in large-scale epidemiological analyses. Given the regional diversity in dietary habits, this study aimed to develop and assess the reproducibility and construct validity of a novel FFQ tailored for northern Karnataka (FFQ-NK).

Methodology: A detailed food list was created based on food use and market surveys in the region. Utilizing data from 24-hour diet recalls and a supplemental focus group discussion, a 116-item semi-quantitative FFQ was developed for adults in north Karnataka.

Setting and Subjects: Involving 100 participants from north Karnataka, the FFQ was interviewer administered and the participants also completed three 24-hour dietary recalls (DR), serving as a reference for validity assessment.

Results: Though the FFQ indicated higher food and nutrient intake compared to food records. Significant correlations were found for nutrient intake. Pearson's correlation coefficients between FFQs and DRs ranged from 0.717-0.965 (isoflavonols to energy).

Conclusions: The developed and validated FFQ is a valuable tool for epidemiological studies requiring nutrient intake estimates in the north Karnataka population. It serves as an effective dietary assessment tool for individuals aged 20 and older in large-scale epidemiological studies.

Key-words: Dietary assessment, food frequency questionnaire, validity, epidemiological studies, north Karnataka

ARTICLE INFO

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***Correspondence:** Dr. Deepa S Sajjanar (Email: deepa.sajjanar@bldedu.ac.in)

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INTRODUCTION

Dietary factors are indeed a significant environmental determinant influencing the development of chronic diseases.¹⁻² The food frequency questionnaire (FFQ) stands out as the predominant tool for assessing nutrient intake in large epidemiological studies, often employed to investigate the link between diet and chronic diseases.³⁻⁴ Additionally, the FFQ facilitates the categorization of subjects into low, medium, and high consumption tertiles, streamlining the exploration of associations with disease prevalence and mortality data in specific populations.⁵ FFQs are cost-effective and practical tools that represent long-term intake of energy, macronutrients, and micronutrients.⁶⁻⁸

They have gained popularity as they pose less tribulation to both the investigator and the subjects in comparison to other dietary assessment methods.⁹⁻¹²

Though the FFQ are not without limitations, they are popular as their feasibility is the major advantage for use in establishing long-term habitual dietary intake.¹³ Given the diverse dietary habits in the Indian population, it is crucial to develop and validate Food Frequency Questionnaires (FFQs) tailored to specific regions and research goals. Dietary patterns, shaped by factors like ethnicity, culture, preferences, and financial status, underline the need for Food Frequency Questionnaire (FFQ) validation against gold standards like specific biochemical markers for precise nutrient intake measurement.¹⁴⁻¹⁶ While biomarkers offer independence from biases, they lack substitution for common dietary components and dietary recommendations. In epidemiological research, FFQs stand out as cost-effective, easily administered tools, surpassing food diaries and biomarkers in utility.¹⁷⁻¹⁹ Despite their widespread use, the less accurate nature of FFQs necessitates data verification against reference techniques, like food diaries, to mitigate biases arising from portion size estimates and memory limitations. The Indian subcontinent is known for its diverse eating habits and the vast variety of foods consumed. The recent transition in the dietary pattern in both traditional food items and with the easy availability of global cuisine has seen a persistent increase in diet-related non-communicable and degenerative diseases.²⁰⁻²²

Dietary instruments like the FFQ play a vital role in epidemiological studies investigating the definitive role of nutrition on lifestyle diseases and formulating health policies. This study addresses the need for a validated FFQ tailored to diverse socio-demographic populations in the Indian subcontinent, particularly north Karnataka. The developed FFQ enables investigations into links between dietary patterns and health outcomes in the north Karnataka population, marking the first of its kind for the region, validated against a three-day 24-hour dietary recall.⁵

The study was motivated by the clear need to develop and validate an FFQ that is suitable to assess dietary

pattern across heterogeneous socio-demographic populations with varying incomes and categories in the regions of Indian subcontinent and of north Karnataka in particular. The study also aimed to explore differences in habitual dietary intakes that were attributed by age, gender, education, social class and body mass index (BMI).

METHODOLOGY

Population setting and cohort recruitment: The FFQ-NK was developed within the diverse population of Vijayapura district in north Karnataka, India, representing both rural and urban demographics. Vijayapura, a prototypical region in north Karnataka, exhibits a dietary pattern centered around staples such as jowar and rice, complemented by a substantial intake of fresh vegetables and greens in the form of curries and spices. Ethical approval for the study was obtained from the Institutional Ethics Committee of BLDE (du) Shri B M Patil Medical College, Hospital, and Research Centre (Ref: BLDE(DU)/IEC/460/2020-21). Participants were provided with detailed information about the study through an information sheet and provided informed consent. The inclusion criteria encompassed healthy individuals above the age of 18, while those on extreme diets (anyone consuming more calories than the specified range (4200 kcal for men and 3500 kcal for women)) or fasting regimens were excluded. A total of 130 participants were recruited by a purposive sampling technique in which the participants were selected as per the maximum variation sampling technique. On the basis of the participant information sheet and after exclusion on the predefined criteria the participants were selected based on urban/rural settings, gender, age group, physical activity and BMI. Additionally, their occupational categories (service and homemakers) and annual income of the household and their educational qualification were also considered but with an intention to ensure that the individuals belonged near equitable representation across the various factors which can affect their dietary choices and patterns.

Development of FFQ-NK: The formulation of the FFQ-NK was meticulously conducted by leveraging information from the National Institute of Nutrition, Hyderabad, India's database, which includes nutritive values of commonly consumed food items.²³ The dietary pattern specific to north Karnataka, including infrequently consumed items, and was derived from a reference database developed for the region.²⁴⁻²⁵

Prior to developing FFQ-NK, extensive literature review, diet surveys, and FFQ development studies were examined. The preliminary stage involved formative fieldwork, which was qualitatively followed by a sequenced mixed-methods approach.²⁶

Food List Construction and Nutrient Alignment:

The development of the FFQ-NK's food list involved a

comprehensive approach, utilizing two primary methods:

- 1. Identification of Food Items:** The food list was compiled through a dual approach: firstly, by identifying commonly consumed local, regional, and global foods using 24-Hour Diet Recalls of 50 representative participants aged 18–60 years. Secondly, written free lists were obtained from a purposive sample of adults, encompassing foods available in local stores and from vendors.²⁷ This combination allowed for a diverse and representative compilation of food items.
- 2. Categorization and Review:** The resulting food list was categorized into fourteen groups based on their ingredients and preparation methods. This categorization facilitated a systematic understanding of dietary patterns within the study population.
- 3. Database Review for Target Nutrients:** To ensure the relevance of the food list to the study's nutritional goals, each listed food item underwent a meticulous review against a comprehensive nutrient database.²⁸ This step aimed to align the food list with the intake of 32 nutrients, including energy, protein, fat, carbohydrate, fibre, various fatty acids (saturated, monounsaturated, polyunsaturated), essential minerals (iron, zinc, magnesium), and a spectrum of vitamins (B1, B2, B6, B12, C, A, D, E), among others.
- 4. Validation of Nutrient Profiles:** A rigorous validation process affirmed that the food list included items accounting for over 95% of the nutrient profiles used for the epidemiological studies.²⁹ This validation was conducted through sample analysis, ensuring that the selected food items sufficiently represented the nutritional diversity of the study population.

Description and the Frequency response section of FFQ-NK: The FFQ-NK was developed with a 116-item scale that measured food consumption over a year (96 composite food items (i.e., food items containing one or more ingredients) and 20 simple food items). The food list was built and categorized into fourteen food groups based on their ingredients and preparation methods: i. Starchy staples ii. Legumes and Nuts iii. Green leafy vegetables iv. Other vegetables v. Egg vi. Flesh foods vii. Milk and dairy products viii. Sweets and desserts, ix. Pickles x. Salads/raw food Fruits xii. Fats and oils xiii. Miscellaneous foods and xiv. Alcoholic beverages

The semi-quantitative FFQ-NK was interviewer-administered to be more precise and had seven frequency categories for food consumption over 1 year, from 2–3 times per day to rarely/never as responses. The participants were also tutored on the normal portion size typically eaten by them. As most of the common fruits are currently available throughout the year, the data capture was done as a single response and was noted as small, medium, and large.

The volume, diameter (2.4 to 3.7 cm) of the most commonly consumed size, and number of pieces were standardized for weight equivalence. They were also assisted in estimating their portion size as a multiple of the standard measure by using metal vessels and spoons, as described in earlier studies.²³

Administration of FFQ: The FFQ-NK was administered to a representative sample of 130 individuals aged 20–70 years. For validation, 3-day, 24-hour diet recall interviews were employed as the reference method due to noted lower overall variance.²⁹ Trained nutritionists conducted open-ended, prompted interviews over three non-consecutive days, ensuring avoidance of days with significant dietary variations. Respondents provided chronological reports of all food encounters throughout the day.

A follow-up response rate of 100 participants was achieved when study's exclusion criteria were applied for data completeness. The resultant tabulation involved multiplying frequency of intakes by portion size and corresponding nutrient profiles reported by participants.

Statistical Analysis: All statistical analyses were conducted using GraphPad Prism (version 9.2, India) for descriptive statistics, frequency distributions, and graphical representations. For advanced statistical modelling and analyses, JMP 16.2 was utilized. The responses from FFQ were subjected to comprehensive analysis, encompassing mean, median, and standard deviation calculations for various nutrients. Descriptive statistics were employed to elucidate demographic details and frequency distribution, providing a foundational understanding of the acquired responses. Prior to analysis, normal distribution tests were conducted, revealing that most variables exhibited normal distribution. However, VIT E, Flavan-3-ol, Flavones, and Flavanones were log-transformed to enhance comparability. To assess the significance across different parameters, regression analysis was undertaken, focusing on gender, domicile, and per capita income. Associations between nutrient intakes from FFQ and the arithmetic mean of the 3-day DR were evaluated using Pearson's correlations. One-way ANOVA tested for overall significance, followed by paired t-tests for specific comparisons. Simple linear regression was applied to assess the linear agreement between nutrient scores obtained from FFQ and the arithmetic average from 3-day DRs. The agreement between the two methods was further evaluated using the Bland-Altman method, offering insights into potential bias, reproducibility and limits of agreement

RESULTS

Demographic characteristics (Table 1): BMI, basal metabolic rate; the study cohort demonstrated a balanced gender representation, with males constituting 51% and females 49%. The majority of partici-

participants fell within the 30–40 years age group and urban residents (56) accounted for the majority of the study population. A substantial portion of participants exhibited a normal BMI (39%), while 47% were classified as overweight. Notably, the majority of individuals (68%) reported engaging in moderate physical activity.

Summary of the nutrient intakes: The summarized findings, presented in Table 2, provide a detailed overview of the statistically significant variations in nutrient intakes across gender, domicile, and per capita income groups. Significant variations in nutrient intake were identified between males and females. Total energy, net carbohydrate, total fat, MUFA, and folate were notably higher in males and the differences were statistically significant and also males reported increased mineral intake, particularly iron and calcium. Distinct differences in nutrient intake were observed between urban and rural dwellers. Notably, disparities in the intake of specific nutrients, including vitamin D, B12, folate and iron, were identified, highlighting the potential influence of domicile on dietary patterns. Participants were stratified based on per capita income levels using Prasad's classification. While the overall mean intake of energy, proteins, vitamin D, vitamin C, and minerals such as iron and calcium appeared lower in the lower economic class (EC) compared to middle and high- economic class (EC), these differences were statistically insignificant, except for vitamin C (P value: 0.04), indicating a potential association between income levels and vitamin C intake.

Validity of the Food frequency questionnaire: The validity of the FFQ was evaluated through Pearson correlations between responses from the FFQ and the mean of three 24-hour DR. The Pearson correlation coefficients were computed for each nutrient,

providing a quantitative measure of the strength and direction of the linear relationship between the FFQ and the 3-day 24-hour DR.

Table 1: Socio-demographic characteristics of study participants (n = 100)

Demography	Participants (%)
Gender	
Male	51 (51)
Female	49 (49)
Age (years)	
<30	24 (24)
31-40	34 (34)
41-50	22 (22)
51-60	12 (12)
>60	8 (8)
BMI (kg/m²)	
Below 18.5	3 (3)
18.5-24.9	39 (39)
25-29.9	47 (47)
30 and above	11 (11)
Per Capita Income/Month (Rs)*	
Below Rs.773 (V)	33 (33)
773-1546 (IV)	20 (20)
1547-2577 (III)	23 (23)
2578-5155 (II)	9 (9)
5156 and Above (I)	15 (15)
No. of family members	
2-4	45 (45)
5-7	42 (42)
8-10	12 (12)
>10	1 (1)
Domicile	
Urban	56 (56)
Rural	44 (44)
Physical activity	
Active	12 (12)
Moderate	68 (68)
Sedentary	20 (20)

* (Prasad modified classification)

Table 2: Mean nutrient intakes across demographic Factors

Nutrients	Male	Female	'p' value	Urban	Rural	'p' value	Lower EC	Middle EC	'P' value
Energy (kcal)	2397	2338	0.00**	2372	2389	0.04	2599	2636	0.87
Carbohydrate(g)	296.9	292.6	0.00**	291.6	298.9	0.17	322.7	307.1	0.62
Proteins (g)	52.6	49.9	0.6	52.0	53.0	0.02	61.6	54.2	0.29
Fat (g)	123.1	132.4	0.005**	122.3	125.5	0.29	133.7	120.6	0.30
Fibre (g)	46.1	44.5	0.63	46.4	49.1	0.12	44.5	48.9	0.50
Saturated fat (g)	37.0	33.8	0.21	37.4	34.1	0.43	36.3	36.5	0.96
Total MUFA(g)	42.8	36.7	0.002**	39.6	40.2	0.53	43.5	40.5	0.43
Total PUFA(g)	39.5	33.0	0.036	35.9	37.2	0.62	41.1	35.6	0.35
Vitamin A (µg)	462	377.2	0.029	417.8	422.1	0.40	466.8	468.3	0.98
Vitamin C (mg)	284.7	346.8	0.086	316.8	320.2	0.19	253.9	375.1	0.04**
Vitamin D(µg)	71.4	67.9	0.377	69.2	67.2	0.00**	69.1	80.5	0.19
Vitamin E (mg)	40.3	38.6	0.850	38.3	41.7	0.78	40.3	43.0	0.87
Vitamin B1 (mg)	3.18	2.89	0.208	3.0	3.2	0.35	3.1	2.9	0.50
Vitamin B2 (mg)	7.92	6.68	0.115	7.2	7.1	0.04	8.0	7.9	0.96
Vitamin B6 (mg)	6.15	5.76	0.338	5.9	6.1	0.20	6.2	5.8	0.63
Vitamin B12 (µg)	1.65	1.71	0.698	1.6	1.7	0.09**	1.62	1.7	0.60
Folate (µg)	358.9	319.3	0.085**	338.7	348.2	0.09**	364.0	332.3	0.47
Iron (mg)	77.1	53.9	0.020**	65.3	66.8	0.00**	73.1	79.4	0.73
Zinc (mg)	7.9	6.0	1.34	6.9	7.0	0.29	8.1	7.3	0.34
Calcium (mg)	440.4	452.1	0.003**	476.2	550.2	0.12	410	447.1	0.043**

SD, standard deviation; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids. Mean nutrient intakes were derived from the initial administration of the FFQ.

Table 3: Correlation Analysis: FFQ vs. 3-Day 24-Hour Dietary Records

Nutrients	FFQ1		DR		Pearson coefficient (Unadjusted (R))	Pearson Coefficient (Energy Adjusted (R))	'p' Value
	Mean	SD	Mean	SD			
Energy (kcal)	2391.2	632.43	2314.41	586.51	.96	—	
Carbohydrates (g)	296.18	84.14	283.25	82.388	.94	0.89	0.14
Proteins (g)	50.99	17.68	48.61	14.57	.92***	0.85	0.00
Fat (g)	121.37	33.91	121.09	33.50	.96	0.91	0.44
Saturated fat (g)	35.51	12.90	35.25	13.41	.96	0.92	0.10
Total MUFA(g)	39.86	10.49	38.79	9.81	.92	0.85	0.35
Total PUFA(g)	36.32	15.66	34.91	15.12	.94	0.88	0.99
Vitamin A(µg)	420.16	198.03	387.97	177.14	.92	0.85	0.92
Vitamin C(mg)	315.58	183.08	304.29	174.25	.78*	0.60	0.08
Vitamin D(µg)	69.72	20.26	68.66	20.39	.92	0.85	0.29
Vitamin E(mg)	39.42	45.87	37.46	43.53	.85***	0.71	0.00
Vitamin B1 (mg)	3.03	1.15	2.89	1.11	.92	0.84	0.19
Vitamin B2 (mg)	7.26	3.99	7.37	3.75	.90**	0.81	0.01
Vitamin B6 (mg)	5.99	2.16	5.717	1.99	.90	0.80	0.42
Vitamin B12(µg)	1.63	.906	1.74	.747	.93	0.86	0.29
Folate (µg)	339.34	116.59	324.87	108.91	.95	0.90	0.51
Total carotenoids-µg	11668.	5069.2	10975.9	4346.6	.94	0.88	0.29
Selenium (µg)	176.49	72.792	174.82	71.881	.76*	0.57	0.08
Omega-6 FA (gm)	47.33	24.772	47.95	24.45	.90*	0.81	0.08
Omega-3FA (gm)	37.92	25.173	39.023	25.01	.86	0.73	0.12
Caffeine(mg)	82.10	53.032	84.72	53.07	.84	0.70	0.12
Magnesium (mg)	878.11	295.24	858.11	281.25	.90	0.90	0.48
Flavan-3-ol(mg)	4.16	2.654	3.56	2.25	.96	0.93	0.66
Flavones(mg)	2.59	2.142	2.35	2.25	.80	0.80	0.81
Flavanols(mg)	7.80	2.981	7.26	3.08	0.77**	0.59	0.00
Flavanones (mg)	6.43	12.440	5.83	12.02	.74***	.54	0.00
Calcium (mg)	440.4	52.1	462.3	63.2	.71	0.50	0.14
Iron (mg)	65.69	50.734	64.21	48.98	.66*	.45	0.08
Fibre (g)	46.08	16.754	42.91	16.59	.90	0.82	0.29
Zinc(mg)	7.05	2.337	6.82	2.18	.87***	0.77	0.00

SD, standard deviation; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids.

Table 4: Associations with reported intake differences: FFQ vs. 3-Day Dietary Record

Nutrients	Age			Sex			Domicile			Per Capita Income		
	R ²	Adj.R ²	P	R ²	Adj.R ²	p	R ²	Adj.R ²	p	R ²	Adj.R ²	p
Energy (kcal)	.039	.029	0.04**	.062	.052	0.01**	1.08	-.001	0.97	0.0579	0.048	0.01**
Carbohydrates(g)	.014	.004	0.22	.008	-.001	0.35	0.00	-.000	0.54	0.0027	-.0007	0.60
Proteins (g)	.002	-.008	0.00**	.034	.024	0.06**	0.01	-.000	0.32	0.0008	-.0009	0.77
Fat (g)	.015	.005	0.00**	.008	-.001	0.35	0.00	-.000	0.63	0.0299	0.019	0.08**
Fibre(g)	0	-.010	0.90	.050	.040	0.02**	0.00	-.000	0.47	0.0100	-.00001	0.32
Total MUFA (g)	2.12	-.010	0.96	.005	-.00	0.46	1.85	-.001	0.96	0.0524	0.042	0.02**
Total PUFA (g)	.015	.005	0.00**	.029	.019	0.09**	0.00	-.000	0.74	0.0524	0.042	0.02**
Saturated fat (g)	.003	-.007	0.00**	.0012	.002	0.27	0.00	-.006	0.56	0.0528	0.043	0.02**
Vitamin A (µg)	.005	-.004	0.46	.000	.00	0.85	0.01	0.00	0.23	0.0413	0.031	0.04**
Vitamin C (mg)	.002	-.007	0.61	2.49	-.01	0.96	0.02	0.01	0.12	0.0135	0.003	0.25
Vitamin D(µg)	.000	-.009	0.77	.008	-.001	0.36	0.00	-.005	0.49	0.0135	0.0033	0.25
Vitamin E (mg)	.012	.002	0.27	.001	-.008	0.69	8.55	-.001	0.92	0.0112	0.0010	0.29
Vitamin B1 (mg)	.017	.007	0.19	.010	.000	0.31	0.00	-.000	0.83	0.0099	-.00002	0.32
Vitamin B2 (mg)	.001	-.009	0.73	.027	.017	0.09**	0.01	0.00	0.28	0.0215	0.011	0.14
Vitamin B6 (mg)	.003	-.006	0.54	.013	.003	0.24	0.00	-.009	0.80	0.0266	0.016	0.10
Vitamin B12 (µg)	.001	-.008	0.71	.000	-.00	0.80	0.00	-.006	0.54	0.0103	0.000	0.31
Folate (µg)	.005	-.004	0.46	.006	-.00	0.43	0.04	0.03	0.04**	0.0007	-.0009	0.78
Iron (mg)	.000	-.010	0.91	.005	-.00	0.46	0.00	-.009	0.81	0.0006	-.0009	0.79
Zinc (mg)	.012	.002	0.00**	.060	.05	0.01**	0.00	-.000	0.53	0.0469	0.037	0.03**
Magnesium (mg)	.012	.002	0.26	.02	.01	0.11	0.0	-.001	0.9	0.03	0.026	0.05**
Total Carotenoids	.0005	-.009	0.82	.010	.00	0.31	0.011	0.001	0.28	0.03	0.028	0.05**

Note: 'R²' and 'Adj. R²' refer to the coefficients of determination and adjusted coefficients of determination, respectively. 'p' denotes the p-values for significance. '0.00' indicates p-values less than 0.001. '**' denotes significance at the 0.01 level, '*' at the 0.05 level. SD, standard deviation; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids.

Positive correlation values indicate a positive linear relationship, while negative values suggest an inverse association. A high positive correlation would indicate strong agreement between nutrient esti-

mates from the FFQ and the 3-day 24-hour DR, implying that the FFQ is a reliable tool for capturing dietary patterns. The correlation analysis serves as a validity assessment, offering insights into how well

the FFQ estimates align with the more detailed information obtained from multiple 24-hour DRs over three days.

Correlation analysis between the FFQ and 3-day DR: The study investigated associations between personal characteristics and the difference in reported nutrient intakes derived from the FFQ and the 3-Day Dietary Record (3-day DR) as shown in table 5. This analysis aimed to identify potential factors influencing variations in reported dietary information. The coefficient of determination (R^2) varied widely, ranging from as low as 0.1% up to 32%, indicating diverse levels of explained variability in reported nutrient intakes between the Food Frequency Questionnaire (FFQ) and the 3-Day Dietary Record (3-day DR). None of the personal characteristics considered, including age, gender, and socio-economic status, were found to be significant in the models for nutrients (lowest R^2 were noted for iron, vitamin C, vitamin D, vitamin B1, vitamin B12, and vitamin B6). Among the personal characteristics, sex emerged as significant for six nutrients, with men exhibiting larger differences in reported intakes for energy, proteins, fibre, PUFA, vitamin B12, and zinc compared to women. Age was found to be significant for intakes of total energy, protein, total fat, saturated fat, PUFA, and zinc. However, the differences were smaller for the intake of carbohydrates, other minerals, and most vitamins. Socio-economic status played a significant role in intakes of energy, total fat, MUFA,

PUFA, saturated fat, vitamin A, magnesium, zinc, and total carotenes. Folate consumption showed a significant increase among the rural population.

Table 5: Bland-Altman Analysis: FFQ vs. 3-Day Dietary record

Nutrient	Bias*	SD	95% CI
Carbohydrates(g)	12.13	27.67	6.64-17.62
Proteins (g)	2.45	7.98	0.87-4.04
Fat (g)	0.99	9.65	-0.91-2.91
Fibre(g)	-2.19	7.61	-0.67-3.7
Total MUFA(g)	1.31	4.01	0.51-2.11
Total PUFA(g)	1.28	5.27	0.23-2.32
Saturated fat (g)	2.92	19.50	-0.42-1.00
Vitamin A (μ g)	12.7	123.6	-11.7-37.27
Vitamin C (mg)	7.21	69.7	-6.61-21.0
Vitamin D(μ g)	0.08	11	-1.37-2.9
Vitamin E (mg)	1.08	18.11	0.03-0.12
Vitamin B1 (mg)	0.14	0.5	0.04-0.24
Vitamin B2 (mg)	-0.03	1.73	-0.38-0.30
Vitamin B6 (mg)	0.27	0.75	0.12-0.42
Vitamin B12 (μ g)	0.07	0.22	0.031-0.120
Folate (μ g)	631.9	221.7	587.8-675.85
Iron (mg)	1.28	21.25	-2.93-5.50
Zinc(mg)	0.29	0.6	0.16-0.43
Magnesium (mg)	11.73	78.48	-3.83-27.30
Total Carotenes(μ g)	552.6	3258.9	-93.9-1199.2

Analysis performed on energy-adjusted nutrient intake; SD, standard deviation; CI, confidence interval; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids. n.s. * Positive differences in the average discrepancy between the two methods indicate an overestimation of nutrient intake by the FFQ.

Figure 1: Bland-Altman Analysis for total energy and macronutrients (protein and carbohydrate): Each participant's (n=100) difference in nutrient intakes between the FFQ (Y-axis) and the average of the 3-day dietary records is plotted against the mean intake from the two methods (X-axis).

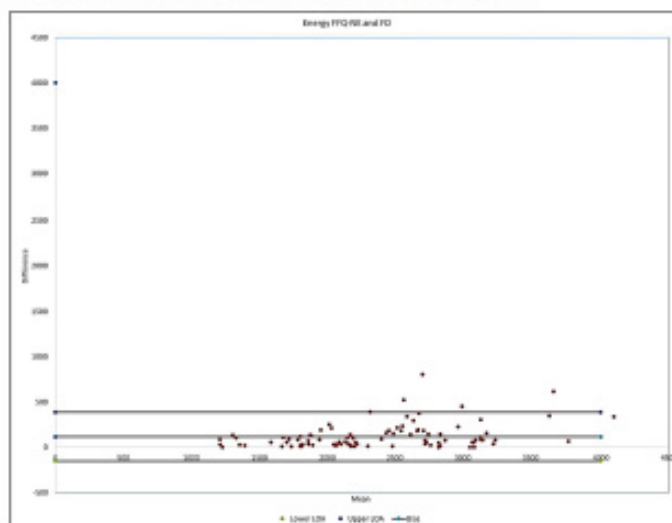


Figure 1(a): Energy (kcal): Mean Difference = 118.0 (95% CI: 90.76-145.24). The Bland-Altman plot for total energy intake shows a mean difference of 118.0, with a 95% CI ranging from 90.76 to 145.24.

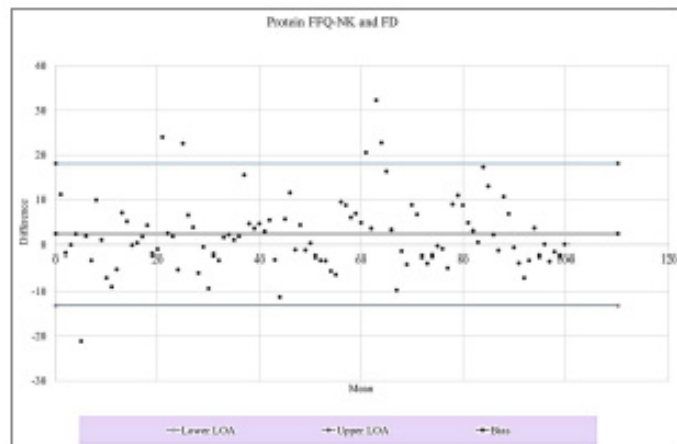


Figure 1(b): Protein (gm): Mean Difference = 2.45 (95% CI: 0.87-4.04). The Bland-Altman plot for protein intake demonstrates a mean difference of 2.45, with a 95% Confidence Interval (CI) ranging from 0.87 to 4.04.

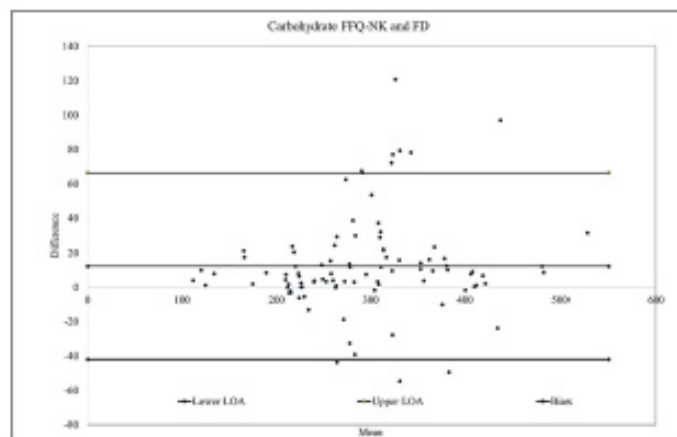


Figure 1(c): Carbohydrate(gm): Mean Difference = 12.13 (95% CI: 6.64-17.62). The Bland-Altman plot for carbohydrate intake reveals a mean difference of 12.13, with a 95% CI spanning from 6.64 to 17.62. The systematic trend indicates that, on average, the FFQ tends to provide higher estimates for protein, carbohydrate, and total energy compared to the 3-day dietary records.

Bland-Altman Analysis: FFQ vs. 3-Day DR: To assess the agreement between reported nutrient intakes from the Food Frequency Questionnaire (FFQ) and the 3-Day DR, a Bland-Altman analysis was conducted. This method provides insights into the extent of agreement, potential biases, and variability between the two dietary assessment tools. Differences in nutrient intake between the FFQ and the 3-day FR were plotted against the mean nutrient intakes of the two methods. Positive differences in the average dis-

crepancy indicated an overestimation of nutrient intake by the FFQ. A larger value of bias, reflected by wider limits of agreement (as indicated by the 95% Confidence Interval), suggested a greater extent of overestimation by the FFQ. For macronutrients such as carbohydrates and fats, as well as a range of micronutrients, there was a bias towards positive differences. This implies that the FFQ tended to provide higher estimates of certain macro- and micronutrients compared to the 3-day food diary. Bland-

Altman plots indicated a systematic trend towards higher estimates with the FFQ for certain nutrients compared to the food diary records. This suggests a consistent pattern of overestimation by the FFQ. The summarized results of the Bland-Altman analysis, including bias, limits of agreement, and other relevant metrics, are tabulated in Table 5.

DISCUSSION

The authors have made a sincere effort to develop an FFQ to capture the dietary pattern, which can be representative of the one widely prevalent among the population of north Karnataka (FFQ-NK), also termed inland north Karnataka (Belgaum, Bagalkot, Bijapur, Gulbarga, Bidar, Gadag, Dharwad and Belary). The pattern can also be extrapolated to the population of nearby regions like inland central Maharashtra (Nanded, Hingoli, Parbhani, Jalna, Aurangabad, Beed, Latur) and inland eastern Maharashtra (Buldana, Akola, Amravati, Wardha and Nagpur), where sorghum is still important in the consumption basket and a staple along with rice and wheat. A conscientious attempt was made to include a comprehensive list of the food items consumed among the sample population, which could be representative for group comparisons as they tend to capture the habitual intake of the population in north Karnataka over a given period. To the best of our knowledge, this 116-item FFQ-NK is the first to be developed and validated for use in population of Karnataka.^{5, 9-11, 26}

The above-validated FFQ-NK shall serve as an important tool to register dietary patterns and average energy and nutrient intake in the region of north Karnataka. It can further serve to design studies that investigate the association between the dietary pattern and common diseases of interest prevalent in this region. Registering energy and nutrient intake is key to obtaining more reliable evidence for diet-health relationships from nutritional cohort studies. In the present study, 100 participants completed all of the questionnaires (i.e., 1 FFQ and three 24-hr dietary recalls). It was validated against a 24-hr diet record as a reference tool, as no truly gold standard exists.²⁹ In comparison with the 24-hour diet record, the FFQ-NK tends to overestimate nutrients unadjusted, as in previous studies.^{8, 22}

Socioeconomic factors like the income of the family also had stronger associations, particularly with the average protein intake and nutrients like vitamin D, vitamin C, calcium, and zinc, than the total energy intake. Age and sex were the other determinants that significantly affected the intake of certain nutrients.

The present study also found that the high- and middle-income sectors had higher intakes of average total energy and most of the micronutrients in comparison to those in the lower income sector. Future researchers should be aware of the likely cumulative impact of the suggested differences and relationships. The majority of research conducted in India

has hitherto generally explored pricing and expenditure as significant determinants of food consumption patterns, with socio-economic and regional factors receiving less attention and as possible determinants of variations in food consumption patterns. Higher income groups are generally linked to healthier eating habits, which include consuming a variety of fruits and vegetables and more sources of proteins like dairy, poultry, meat, and oil. People in higher income levels tend to consume more of these foods than those in lower income classes, likely because higher income levels correlate with higher socioeconomic standing, which raises awareness of health issues and nutritious dietary options.²⁰⁻²²

Moreover, studies have also reported that low purchasing power, either due to a lack of regular employment or lower socio-economic status, is another possible factor for the lower consumption, as is also the lack of education about the nutritional benefits of fruits and vegetables.²³⁻²⁵

The average of the 3-day DR was considered to correlate with the FFQ derived nutrient values for validation. The correlation coefficient (energy adjusted) that was discerned between the FFQ and 3-day DR (0.45 to 0.93) was comparable to those reported by similar validation studies conducted in Trivandrum, South Kerala (0.34 to 0.72)²⁵ and also by studies set in rural villages in Ernakulam district, Kerala (ranging from 0.32 to 0.61)⁵ and in Gujarat (ranging from 0.55 to 1.00).⁹ Additionally, some other studies have also demonstrated a range of coefficients that appeared to be similar to our range.^{26,28,29} The findings of this study indicate that macronutrients like total fats and proteins have the largest association, whereas trace elements like iron, selenium, and calcium and vitamins like vitamin C have lesser correlations.^{27,28} and the flavonols (0.59) and flavanones (0.54) also had lesser correlations. As previously established, these nutrients may tend to have weaker correlation coefficients and significant within-person variability in validation studies since they are not concentrated in most diets.^{5,27}

Certain macronutrients, such as carbohydrates and proteins, and others, such as folate, total carotenoids, zinc, iron, selenium, and magnesium, were overestimated by the FFQ compared to the 24-hour dietary record. Also, vitamins like A, D, E, C, B1, and B6 were overestimated by the FFQ, whereas vitamins B2 and B12, along with copper and zinc, were underestimated by the FFQ compared to the food record. It is noted that the independent sociodemographic factors may account for 32% of the variation in the difference between the two assessment techniques. For the majority of them, gender was a significant explanatory factor, with men over reporting intakes in comparison to women for total energy, proteins, fibre, PUFA, vitamin B12, and zinc, whereas other researchers noted a reportedly increased intake by women for most of the nutrients.²⁹ Dietary fibre intakes have been linked to lower family incomes in previous studies⁴⁰ that looked at the relationship be-

tween income and dietary fibre intake. Similarly, our findings discerned an increased intake of dietary fibre by the middle- and higher-income groups and those belonging to the rural setting. Apart from being the first to develop a FFQ for use in the north Karnataka population, our study has some significant strengths. First, a wide range of tests were used to assess the validity of the FFQ, correlation coefficients in conjunction with the Bland-Altman approach. When assessing the validity and reproducibility of a food frequency questionnaire, the Bland-Altman method has been found to be more effective than correlation analysis. Furthermore, the study's sample size was adequate to estimate the limits of agreement for the Bland-Altman analysis, which is a prerequisite for evaluating the validity of the FFQ.²⁹

The FFQ was administered by the interviewers, who were registered and experienced nutritionists practicing at the institute, and we believe that this is the study's strength preventing underreporting and enhancing consistency. The participants were also trained to understand portion sizes. The guidance on portion size will be implemented as a pre-requisite for all future administrations of the current validated FFQ. A 116-item FFQ could have been lengthy, which may have led to participant enervation and also nutrient overestimation at times. Some of the recognized sources of error we would like the researchers to be wary of are predetermined and set food lists, intra-individual variations due to memory and interview fatigue, and also errors in interpreting the questions and portions of the food items listed.⁴¹

Additionally, though DR and 24-hour food diaries are often used as the most accepted reference tools for validating the FFQs, they are not known to be without limitations due to reporting errors attributable to gender and differences in BMI of the individuals.²⁶ Omission of validated biomarkers for nutrient assessment represents a notable limitation in our study, impacting the comprehensive validation of nutrient intake data. The absence of direct biomarker measurement may affect the accuracy and reliability of nutritional assessments, warranting cautious interpretation of the results. Future researchers can strengthen the findings of the current study by including biomarkers specific to the nutrients for cross-validation studies. In summary, the creation and validation of the present Food Frequency Questionnaire (FFQ) constitute a critical first measure that enables us to employ it as a key instrument in epidemiological research to evaluate food intakes, dietary patterns, and associations with disease attributes in the people of North Karnataka, India. It would be wise to evaluate the prospects of the application of this FFQ in the regions of north Karnataka where the populace has similar dietary habits.

CONCLUSION

In summary, the authors have successfully developed and validated the first Food Frequency Question-

naire (FFQ-NK) for the population of north Karnataka, India, providing a valuable tool for assessing dietary patterns and nutrient intake in the region. The FFQ-NK, comprising 116 items, demonstrates associations with socioeconomic factors, age, and sex, highlighting disparities in nutrient intake among different income groups. Despite certain limitations, such as potential overestimation and reliance on self-reporting, the FFQ-NK offers a robust foundation for future epidemiological research in understanding dietary influences on health in this specific population.

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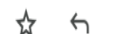
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to me ▾

Dear **Dr Deepa Sanjeev Sajjanar**

A manuscript entitled : **Comparative Analysis of Dietary Inflammatory Index, Tumor Necrosis Factor-Alpha, and Metabolic Parameters in Diabetic and Non-Diabetic Coronary Artery Disease Patients in North Karnataka**. with the manuscript id: **JIMA11112737** has been accepted by the *Editor* and is ready to publish.

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