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Integrated Assessment of Anthropometric, Biochemical, Inflammatory and Nutritional Biomarkers for Cardiometabolic Risk Among Voluntary Employees

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ABSTRACT: The increasing prevalence of obesity, type 2 diabetes mellitus (T2DM), metabolic syndrome and cardiovascular disease (CVD) among the working population is often considered to be influenced by lifestyle and work-related activities such as physical inactivity, unhealthy eating habits, prolonged stress and mental overload. Micronutrient deficiencies have now emerged as a critical factor in modulating metabolic disturbances affecting adiposity, glycaemic regulation and chronic low-grade systemic inflammation. The aim of this study was to explore the association between serum concentrations of various micronutrients like zinc, calcium, iron, magnesium, vitamin D and vitamin B12 with body mass index (BMI), glycated haemoglobin (HbA1c) and high sensitivity C-reactive protein (hs-CRP) in apparently healthy working employees. Two hundred participants were enrolled in the present cross-sectional study. Participants underwent anthropometric assessment including BMI, waist circumference, hip circumference, waist-to-hip ratio and blood pressure measurement. The serum concentrations of the analysed micronutrients, HbA1c, hs-CRP and some routine biochemical parameters were determined on an automated clinical chemistry analyser. The mean BMI was 26.84 ± 4.92 kg/m², indicating that a substantial proportion of the participants were overweight. Average fasting blood glucose was 119.84/66.99 mg/dL and average HbA1c level was 6.31 1.65%. Deficiencies of vitamin D and marginal deficiency of zinc were the predominant micronutrient derangements. Serum iron, calcium, magnesium and vitamin B12 concentrations exhibited considerable inter-individual variability. Our results showed significant association of elevated BMI with increased hs-CRP level and low concentration of vitamin D. Thus, it suggests there may be some connection between these micronutrient abnormalities, excess adiposity and chronic low-grade inflammation. Although a few haematological indices remained outside the normal reference range, the observed findings suggest that even in apparently healthy individual's subclinical micronutrient deficiency could predispose them to developing early cardiometabolic abnormalities. These findings support incorporating anthropometric, biochemical, inflammatory and micronutrient assessments into routine occupational health screening programmes for early identification of individuals at increased cardiometabolic risk.

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1. INTRODUCTION

In recent decades, the worldwide increase in the prevalence of NCDs including obesity, type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVDs), hypertension and metabolic syndrome have presented a serious public health challenge [1]. NCDs are responsible for approximately 75% of all deaths globally and cause an estimated loss in the value of world output of 14% per year over the next 15-20 years, with developing countries estimated to lose over 4.5% of GDP per year (WHO 2015). Obesity, impairment in glucose homeostasis and a state of chronic low-grade inflammation have been proposed as major underlying features of cardiometabolic diseases and the identification of subjects with increased risk prior to clinical manifestations is therefore warranted [2]. Rapid urbanization, technological advancements and occupational transitions have dramatically reshaped the lifestyles of working populations. An increase in sedentary behaviour, leisure activities, poor diet quality, chronic psychosocial stress, lack of sufficient sleep, among other lifestyle-related factors, have contributed to higher rates of obesity, insulin resistance, dyslipidaemia, systemic inflammation and consequently cardiometabolic disease [3,4].

The majority of these metabolic perturbations remain subclinical and may not be detectable by routine clinical examination for several years before they progress to overtly evident diabetes or cardiovascular disease. This makes workplace-based health screening programs an effective strategy for early cardiometabolic risk detection. Anthropometric measures including body mass index (BMI), waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), and blood pressure are among the widely adopted reliable, cost-effective and simple methods for estimating the risk of obesity and cardiovascular events [5]. Among anthropometric measures, BMI is the most widely utilized indicator of general obesity, while WC and WHR are better indicators of central adiposity, which is strongly correlated with insulin resistance, metabolic syndrome, hypertension and cardiovascular disease [6]. Fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) are essential biochemical tests that provide information on glucose regulation. HbA1cis used for the diagnosis of T2DM and the long-term management of the condition as it reflects the average blood glucose levels over the prior 2-3 months and it is considered as the current gold-standard biomarker for diagnosis [7]. Low-grade chronic inflammation is an important mechanistic link among obesity, insulin resistance and cardiovascular disease. C-reactive protein measured with high-sensitivity (hs-CRP), an acute phase protein synthesized by the liver, is a sensitive and widely used marker of systemic inflammation. Elevated hs-CRP levels are routinely associated with increased adiposity, insulin resistance, endothelial dysfunction, metabolic syndrome, and increased cardiovascular risk, and its elevation often occurs at early stages of cardiovascular disease processes [8, 9]. Thus, hs-CRP serves as a valuable early marker of risk among apparently healthy subjects. It is also recognized that deficiencies in several micronutrients contribute to the genesis and pathogenesis of cardiometabolic diseases. For instance, vitamins (such as vitamins B12, D) and minerals (suchaszinccalcium, iron, magnesium) play critical roles in regulating many aspects of glucose and lipid metabolism, insulin signalling, immune function, antioxidant defense, inflammatory processes, cellular energetics, mitochondrial function, etc [10].

Among the micronutrients listed, Vitamin D deficiency is the one of the most common nutritional disorders worldwide and is well known to be associated with increased adiposity, reduced insulin sensitivity, endothelial dysfunction, chronic inflammation, and higher risk of cardiovascular diseases. Likewise, deficiency in Vitamin B12 is associated with impaired one-carbon metabolism, hyperhomocysteinaemia, neurological disorders, and glucose metabolism [11]. Essential minerals like Zinc, magnesium, calcium and iron play a key role in metabolic homeostasis. Zinc is required for insulin synthesis and secretion and is an antioxidant. Magnesium is required as cofactor for several carbohydrate metabolic enzymes and for insulin signalling. Calcium regulates cellular signalling, insulin-mediated glucose transport while iron is necessary for transport of oxygen, and cellular energy production in mitochondria [12]. Iron deficiency has clinical implications for cardiovascular health especially because iron deficiency can damage the heart's mitochondria and lower the ATP that the heart needs to contract. Iron deficiency is common in people who have heart failure even when they do not have anaemia and iron deficiency brings fatigue, ability to exercise poorer heart function and a higher chance of being admitted to hospital. Anaemia lowers the oxygen that reaches heart tissue making the heart beat faster and pump blood to try to compensate [13,14]. On the hand polycythemia can thicken the blood and raise resistance in blood vessels, which also makes the heart work harder. Continual problems with iron status and oxygen delivery can cause the heart to remodel lead to ventricular trouble and worsen disease. For these reasons finding iron deficiency early and treating it properly can be very important for keeping heart muscle working and, for better cardiovascular results. Furthermore, individuals that showed higher hs-CRP and vitamin D deficit tended to present lower values for serum iron and hint at possible interaction between inflammation micronutrient deficiency

and metabolic disorder. Hence, imbalance in micronutrient homeostasis may aggravate obesity induced inflammation and metabolic abnormalities, leading to an increased risk of developing cardiometabolic disorders [15].

The effects of single nutrient deficiencies on Cardiometabolic disorders have been well explored in different study groups. However, comprehensive studies simultaneously evaluating multiple serum micronutrients together with cardiometabolic biomarkers among apparently healthy working adults remain limited, particularly in the Indian population [16]. Moreover, simultaneous evaluation of serum Zinc, Calcium, Haemoglobin, Iron, Magnesium, Vitamin D and Vitamin B12 levels with BMI, HbA1c, and hs-CRP in apparently healthy individuals and workers are scant in literature. Information about such associations will allow the screening of healthy workers at high risk of developing the same in a primary health care setting, which would also help in timely nutritional intervention [17]. Thus, the objective of the present study was to find out the association of serum micronutrients status (Zinc, Calcium, Iron, Magnesium, Vitamin D and Vitamin B12) with cardiometabolic parameters like Body mass Index (BMI), HbA1c, and High-sensitivity C-reactive Protein (hs-CRP) inapparently healthy working employees. Such data is expected to add useful evidence for the inclusion of micronutrient evaluation in standard occupational health check-ups to predict cardiometabolic diseases [18].

2. MATERIALS AND METHODS

2.1 Study Design and Study Population

A cross-sectional observational study was performed to determine the correlation between the serum micronutrient status and the cardiometabolic markers in 200 apparently healthy working employees from an organization. We assessed data collected through a routine occupational health screening program. The main aim of our study was to assess the relationship between serum levels of micronutrients such as calcium, iron, zinc, magnesium, vitamin D and vitamin B12 with anthropometric markers and cardiometabolic markers such as BMI, HbA1c and hs-CRP. Both healthy male and female employees of the age of 18 and above, who had volunteered to attend the occupational health screening were selected. We excluded participants with evidence of infection, pregnant women, people with chronic inflammatory condition, malignancy, or severe hepatic, renal or endocrinal diseases and any subjects with incomplete medical or laboratory information. Anthropometric measures, clinical data and biochemical analysis results as well as information on age and gender were collected from the anonymized occupational health record files of the participating individuals after deleting personal identification. All ethical principles stated in the Declaration of Helsinki were complied.

2.2 Anthropometric and Clinical Assessment

Anthropometric and clinical measurements were conducted by trained clinical personnel in accordance with standard operating procedures (SOPs) and according to Good Laboratory Practice (GLP) standards. Data such as the age and sex were obtained from each of the participants. Anthropometric measures obtained were: height (cm), body weight (kg), waist circumference (WC, cm), hip circumference (HC, cm), body mass index (BMI), and waist to hip ratio (WHR). The stadiometer was used for measurement of height, and a calibrated digital weighing balance (on which individuals were weighed with their shoes off and minimal clothing) was used for measurement of weight. Body mass index (BMI) was calculated as: Weight (kg) Body mass index =_ [2] Height (m) Waist to hip ratio was calculated as: Waist Circumference Waist to hip ratio= [3] Hip Circumference Waist circumference measured midway between the lower border of the costal margin and the crest of ilium by means of a non-stretchable measuring tape; hip circumference was measured around the maximal extent of the buttock. The Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) of participants were measured by using a calibrated automatic digital sphygmomanometer after participant was at rest for not less than five minutes. These anthropometric and clinical variables were determined as the indicator of overall general and central adiposity, to assess relationship with serum concentration of micronutrient and cardio metabolic markers.

2.3 Blood Collection and Biochemical Profiling

Following an 8 to 12-hour fast, about 5 mL of venous blood was aseptically collected from each subject. Samples for serum biochemical analyses were collected in plain vacutainer tubes, while samples for Complete Blood Counts (CBC), HbA1c determination were collected in EDTA tubes, test assessed in Sysmex XP100 for CBC and BioRad D10 for HBA1C. Separated serum was obtained by centrifugation at 3000 rpm for 10 minutes, and analysed immediately or stored under the required conditions until analysis. All laboratory examinations were performed in an accredited clinical diagnostic laboratory

(Randox Daytona Plus) that participates in internal and external quality assurance. The primary biochemical parameters evaluated include fasting blood glucose (FBG), glycated haemoglobin (HbA1c), high sensitivity C-reactive protein (hs-CRP), calcium, iron, zinc, magnesium, vitamin D, and vitamin B12. All parameters were analyzed with fully automated clinical chemistry and immunoassay analysers (Abbott Architect SRi1000(Vitamin D, B12), in accordance with the manufacturer's instructions and the standard operating procedures of the laboratory. The routine biochemical tests to screen metabolic profile of participants included lipid profile (total cholesterol, triglycerides, HDL, LDL, and VLDL cholesterol), liver function tests (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, albumin, and globulins) and renal function tests (blood urea nitrogen, serum creatinine, and uric acid).

2.4 Inflammatory, Nutritional and Haematological assessment

High-sensitivity C-reactive protein (hs-CRP) was quantified by high-sensitivity immunoturbidimetric assay on the Randox Daytona Plus analyser in accordance with the manufacturer's instructions. Hs-CRP, a sensitive indicator of systemic low-grade inflammation that is known to be correlated with obesity, insulin resistance and cardiovascular disease, was included as an inflammatory biomarker. Vitamin D (25-hydroxyvitamin D), vitamin B12, zinc, iron, calcium and magnesium serum levels were measured with fully automated clinical chemistry and immunoassay analysers by following laboratory standardised procedures. Selected micronutrients were investigated because of their documented influence on insulin signalling, glucose metabolism, the immune system, antioxidant defense, inflammation and metabolic balance. Basic routine haematological parameters were measured by an automated haematology analyser Sysmex XP100 and included haemoglobin (Hb), red blood cells count (RBC), white blood cells count (WBC), platelet count, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) and differentials count (neutrophils, lymphocytes, monocytes, eosinophils, basophils).

2.5 Statistical Analysis

Prior to statistical analyses, the dataset was checked for accuracy and completeness. Continuous data was summarised as mean ± standard deviation (SD), and categorical data were reported as frequencies (and their percentages). Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA), and the graphs were created using GraphPad Prism version 10.0 (GraphPad Software Inc., San Diego, CA, USA). The normality of continuous data was determined using Shapiro-Wilk test. Pearson's correlation coefficient (r) was calculated to investigate correlations among anthropometric variables including BMI, and the serum levels of micronutrients including zinc, calcium, iron, magnesium, vitamin D, vitamin B12, HbA1c, and hs-CRP. Correlations were also assessed between Haemoglobin, HbA1c and hs-CRP. Descriptive statistics (means, standard deviations, min, max) for all study variables were computed. Two-tailed P-value<0.05 was considered significant. The statistical methods were conducted to describe the micronutrient status of subjects and assess association of serum micronutrient concentrations with cardiometabolic biomarkers to facilitate early detection of cardiometabolic risk in apparently healthy working employees.

3. RESULTS

3.1. Demographic and anthropometric features of the study group

In this cross-sectional study, a total of 200 apparently healthy working employees participated. There were 84 males (42.0%) and 116 females (58.0%) in the study population and the ages of the participants varied from 21 years to 75 years with a mean age of 44.87±11.94 years, which shows a predominantly middle-aged workforce. Table 1 lists the basic demographic and anthropometric characteristics of the participants in this study.

Participants had mean height and weight of 159.42±8.67 cm and 68.21±13.52 kg, respectively. The mean body mass index (BMI) of the participants was 26.84±4.92 kg/m² (17.60-41.80 kg/m²), indicating that more than half of the participants were in the overweight category of WHO BMI classification system. Participants' mean waist circumference, hip circumference, and waist to hip ratio were 89.36±11.24 cm, 98.74±9.63 cm, and 0.90±0.07, respectively, which is suggestive of a considerable group with abdominal obesity among the study participants. Similarly, the participants showed a mean fasting blood glucose (FBG) and HbA1c level of 119.84±34.26mg/dL (76.00-286.00 mg/dL) and 6.02±1.18% (4.70-10.80%), respectively, suggestive of a compromised glycaemic profile in a proportion of the participants even if they presented with no evident symptoms or clinical history of any illness. Taken together, demographic and anthropometric data indicates a wide

range of age, body mass composition, and glycaemic level among study participants and it has been used as the basis to examine the association of serum micronutrient status with cardio-metabolic indicators such as BMI, HbA1c, and hs-CRP.

Table 1. Demographic and Anthropometric Characteristics of the Study Population (n **= 200)**

Parameter	Mean ± SD / n (%)	Range
Number of participants	200	–
Male	84 (42.0%)	–
Female	116 (58.0%)	–
Age (years)	44.87 ± 11.94	21–75
Height (cm)	159.42 ± 8.67	142.00–182.00
Weight (kg)	68.21 ± 13.52	41.50–108.60
Body Mass Index (kg/m²)	26.84 ± 4.92	17.60–41.80
Waist Circumference (cm)	89.36 ± 11.24	66.00–124.00
Hip Circumference (cm)	98.74 ± 9.63	79.00–128.00
Waist-to-Hip Ratio	0.90 ± 0.07	0.72–1.08
Systolic Blood Pressure (mmHg)	124.68 ± 13.74	98–168
Diastolic Blood Pressure (mmHg)	79.82 ± 8.95	60–104
Fasting Blood Glucose (mg/dL)	119.84 ± 34.26	76.00–286.00
HbA1c (%)	6.02 ± 1.18	4.70–10.80

Values expressed as mean ± standard deviation (SD) unless otherwise stated. BMI: Body mass index; HbA1c: Glycated haemoglobin. Anthropometric measurements were taken following standard techniques as part of occupational health screening.

3.2 Biochemical Characteristics of the Study Population

Table 2 summarizes the biochemical characteristics of the study population. Blood samples collected were analyzed for FBG, HbA1c, high-sensitivity C-reactive protein (hs-CRP) and selected serum micronutrients relevant to cardiometabolic health: iron, calcium, magnesium, zinc, vitamin D, and vitamin B12.The mean FBG of the participants was 119.84 34.26 mg/dL, and the mean HbA1c level was 6.02 1.18%. According to American Diabetes Association (ADA) criteria, many participants already demonstrated signs of abnormal glycemic regulation despite their outwardly normal health on occupation health screening examination. These findings implied the existence of early disturbances in glucose metabolism of the population group.

The mean serum concentrations of iron, calcium, magnesium, zinc, vitamin B12 and vitamin D were 71.52 26.47 g/dL, 9.41 0.62 mg/dL, 2.18 0.21 mg/dL, 82.14 16.58 g/dL, 286.45 118.64 pg/mL and 18.74 7.32 ng/mL, respectively.

Table 2. Biochemical Characteristics of the Population Study (n = 200)

Parameter	Mean ± SD	Reference Range
Fasting Blood Glucose (mg/dL)	119.84 ± 34.26	70–99
HbA1c (%)	6.02 ± 1.18	<5.7
hs-CRP (mg/L)	4.96 ± 3.71	<3.0
Serum Iron (µg/dL)	71.52 ± 26.47	60–170

Serum Calcium (mg/dL)	9.41 ± 0.62	8.5–10.5
Serum Magnesium (mg/dL)	2.18 ± 0.21	1.7–2.4
Serum Zinc (µg/dL)	82.14 ± 16.58	70–120
Vitamin D (ng/mL)	18.74 ± 7.32	≥30
Vitamin B12 (pg/mL)	286.45 ± 118.64	200–900
Total Cholesterol (mg/dL)	186.74 ± 34.82	<200
Triglycerides (mg/dL)	148.63 ± 52.14	<150
HDL Cholesterol (mg/dL)	47.82 ± 10.34	>40
LDL Cholesterol (mg/dL)	108.36 ± 28.74	<130
VLDL Cholesterol (mg/dL)	29.73 ± 10.43	5–40
ALT (U/L)	28.64 ± 12.18	7–56
AST (U/L)	24.18 ± 9.47	10–40
ALP (U/L)	92.54 ± 28.36	44–147
Blood Urea Nitrogen (mg/dL)	15.81 ± 4.32	7–20
Serum Creatinine (mg/dL)	0.89 ± 0.18	0.6–1.3
Uric Acid (mg/dL)	5.42 ± 1.31	3.5–7.2

Data presented as mean ± SD. HbA1c = Glycated haemoglobin, hs-CRP = High-sensitivity C-reactive protein, HDL = High-density lipoprotein, LDL = Low-density lipoprotein, VLDL = Very-low-density lipoprotein, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase.

Vitamin D deficiency was the most common micronutrient deficiency, with a large proportion of the participants having serum vitamin D below the normal reference range. Inconsistent concentrations in serum vitamin B12, iron and zinc were observed.

Mean serum hs-CRP was 4.96 3.71 mg/L, which reflects a general inflammatory response of the body to possibly the prevailing problem of overweight/obesity in the group, though most were outwardly appearing healthy and free of overt symptoms. Most of routine biochemistry findings such as lipid profile, liver functions test and renal functions tests were within normal limits. Thus, they were less likely to cause significant confounders to the observed micronutrient relationships.

The general picture of biochemistry of outwardly healthy employees revealed presence of initial abnormalities of metabolic parameters, e.g., subclinical glycaemic abnormalities, vitamin D deficiency and low-grade chronic systemic inflammation and variation of essential micronutrients. The present data suggest the value of incorporating biochemical and micronutrient assessment in routine occupation health screenings of employees as a preliminary tool to identify employees at high risk of future cardiometabolic diseases.

3.3 Inflammatory, Nutritional, and Haematological Profile

The inflammatory, nutritional, and haematological profile of study participants is shown in Table 3. Circulating high-sensitivity C-reactive protein (hs-CRP), vitamin D, vitamin B12, iron, calcium, magnesium, and zinc were selected and measured because they have demonstrated impact on metabolic regulation, inflammation, immune responses, and cardiometabolic health. The average serum hs- CRP was 4.96 ±3.71 mg/L (range 0.20-18.80 mg/L) indicating wide inter-subject variation. A high percentage of the participants displayed values higher than the standard laboratory reference ranges for the hs-CRP, suggestive of underlying chronic, systemic inflammation that is now widely accepted as a mediator in the development of obesity, insulin resistance, the metabolic syndrome, and cardiometabolic disease.1 The average serum vitamin D was 18.74 ±7.32 ng/mL, which suggests that deficiency of vitamin D continues to be common.

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A total of29.5% (59/200) and 21.0% (42/200) of participants were classified as having either severe or moderate deficiency in their plasma vitamin D concentrations respectively. The average plasma vitamin B12 level was 286.45 ±118.64pg/mLwith marked variation among the individuals, as expected due to diet and other physiological factors. Similar moderate variation among subjects were observed in plasma levels of iron, calcium, magnesium, and zinc. The primary intent of measuring micronutrient concentrations was to assess their relation to the cardiometabolic indices in the present study. Standard haematological assessments including red blood cell count (RBC), white blood cell count (WBC), platelet count (PC), haematocrit, RBC indices and differential leukocyte counts were mostly within acceptable laboratory reference ranges for the subjects in our sample. All of these measurements are standard for screening purposes and indicated that there were no clinically relevant haematological disturbances in the vast majority of healthy working individuals. Overall, in the context of vitamin D deficiency and other varied concentrations of micronutrients in a seemingly healthy working population, the co-existence of elevated hs-CRP values hints that subclinical nutritional imbalances and chronic low-grade systemic inflammation might be playing an early role in the pathophysiology of cardiometabolic disturbances.

3.3.1 Haematological Profile of the Study Population

Table 3 shows the haematological profile of the study participants. Generally, most of the Complete Blood Count (CBC) results of the participants were within their normal reference ranges provided by the laboratory, indicating no major abnormality of the haematological parameters. Mean Hb concentration was 12.41 ± 1.68 g/dL. The mean RBC and WBC count was 4.51 ± 0.52 ×10/L and 7,962 ± 1,864 cells/L respectively. The mean platelet count was 284.16 ± 62.84 ×10/L which was within normal limits. The mean haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW) values were 38.72 ± 4.36 %, 86.94 ± 5.82 fL, 28.41 ± 2.14 pg, 32.68 ± 1.21 g/dL and 13.42 ± 1.26 % respectively. Differential count shows mean percentage of neutrophil as 60.84 ± 8.74%, lymphocyte 31.92 ± 7.21%, monocyte 5.82 ± 1.84%, eosinophil 1.92 ± 1.16% and basophil 0.50 ± 0.28%. Though a Most of participant's had haemoglobin some results slightly below the range, indicating low level of iron, which are correlated biochemical parameters also. None of the other participants had consistently high or low results of any of the haematological parameters to indicate significant clinical disorders. In summary, none of the participants in the study had significant haematological disease. Therefore, it is not possible that any alteration of BMI, HbA1c, hs-CRP and serum micronutrients concentration resulted from underlying haematological disorder(s) of participants and thus strengthens our current associations of findings in this study.

Table 3. Inflammatory, Nutritional and Haematological Profile of the Study Population **(n = 200)**

Parameter	Mean ± SD	Range
Inflammatory Markers		
hs-CRP (mg/L)	4.96 ± 3.71	0.20–18.80
Micronutrient Profile		
Vitamin D (ng/mL)	18.74 ± 7.32	6.20–42.80
Vitamin B12 (pg/mL)	286.45 ± 118.64	108.00–682.00
Iron (µg/dL)	71.52 ± 26.47	28.00–156.00
Zinc (µg/dL)	82.14 ± 16.58	48.00–128.00
Calcium (mg/dL)	9.41 ± 0.62	8.10–10.70
Magnesium (mg/dL)	2.18 ± 0.21	1.62–2.74
Vitamin D Status		
Severe deficiency (<10 ng/mL)	59 (29.5%)	–
Moderate deficiency (10–20 ng/mL)	42 (21.0%)	–

Mild deficiency (20–30 ng/mL)	53 (26.5%)	–
Sufficient (>30 ng/mL)	46 (23.0%)	–
Haematological Parameters		
Haemoglobin (g/dL)	12.41 ± 1.68	8.90–16.70
RBC ($\times 10^6$/μL)	4.51 ± 0.52	3.40–5.82
WBC (cells/μL)	7,962 ± 1,864	4,100–13,800
Platelet Count ($\times 10^3$/μL)	284.16 ± 62.84	156–468
Haematocrit (%)	38.72 ± 4.36	29.60–49.80
MCV (fL)	86.94 ± 5.82	73.80–99.60
MCH (pg)	28.41 ± 2.14	22.40–34.10
MCHC (g/dL)	32.68 ± 1.21	30.10–35.80
RDW (%)	13.42 ± 1.26	11.10–16.70
Neutrophils (%)	60.84 ± 8.74	41–78
Lymphocytes (%)	31.92 ± 7.21	16–48
Monocytes (%)	5.82 ± 1.84	2–11
Eosinophils (%)	1.92 ± 1.16	0–6
Basophils (%)	0.50 ± 0.28	0–2

Values are means ± standard deviation (SD) or number (%). Hs-CRP, high-sensitivity C-reactive protein; RBC, red blood cells; WBC, white blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red cell distribution width.

3.4 Correlation between Serum Micronutrients and Cardiometabolic Biomarkers

Biomarkers Pearson's correlation analysis was performed to examine the relationship between serum micronutrient levels, anthropometric indices and cardiometabolic markers. Correlation coefficients (r) and p-values are given in Table 4. With respect to anthropometric indices, body mass index (BMI) showed a significant positive correlation with high-sensitivity C-reactive protein (hs-CRP) ($r = 0.431$, $p < 0.001$), suggesting an increase in systemic inflammation with increasing obesity. However, BMI was significantly negatively correlated with serum vitamin D ($r = -0.384$, $p < 0.001$), which suggests that subjects with higher BMI had lower concentrations of circulating vitamin D.

This is consistent with previous findings that obesity is related to chronic inflammation and decreased vitamin D bioavailability. There was a weak but significant positive correlation between BMI and HbA1c ($r = 0.262$, $p = 0.001$), suggesting an impaired glycaemic control with increasing BMI. Additionally, HbA1c was significantly positively correlated with hs-CRP ($r = 0.316$, $p < 0.001$), which shows an association between chronical hyperglycaemia and systemic inflammation. With regard to the selected micronutrients, there was a weak but significant negative correlation between serum magnesium ($r = -0.154$, $p = 0.029$) and serum zinc ($r = -0.181$, $p = 0.011$) and BMI. There were no significant correlations between serum iron ($r = -0.097$, $p = 0.174$), calcium ($r = -0.086$, $p = 0.228$), vitamin B12 ($r = -0.104$, $p = 0.143$) and BMI. Similarly, no significant correlations were observed between these micronutrients and hs-CRP or HbA1c ($p > 0.05$). Iron has seen low level in most of the patients who has elevated HsCRP and HBA1C.Overall, the correlation analysis indicated a significant positive association between increased adiposity, inflammation and impaired glycaemic control as well as a reduced vitamin D status in the participants. These results suggest that a vitamin D deficiency, accompanied by an increase of hs-CRP levels, might represent early markers for cardiometabolic abnormalities associated with obesity in seemingly healthy working

individuals. Iron also can be indicative for heart disease. Variations in concentrations of the other micronutrients further support the rationale for a detailed nutritional assessment within occupational health screenings programmes.

Table 4. Pearson's Correlation between Serum Micronutrients and Cardiometabolic Biomarkers (n = 200)

Variables	Correlation coefficient (r)	p-value
BMI vs hs-CRP	0.431	<0.001*
BMI vs Vitamin D	−0.384	<0.001*
BMI vs HbA1c	0.262	0.001*
BMI vs Fasting Blood Glucose	0.118	0.096
BMI vs Zinc	−0.181	0.011*
BMI vs Magnesium	−0.154	0.029*
BMI vs Iron	−0.097	0.174
BMI vs Calcium	−0.086	0.228
BMI vs Vitamin B12	−0.104	0.143
HbA1c vs hs-CRP	0.316	<0.001*
Vitamin D vs hs-CRP	−0.278	<0.001*

Values are Pearson's correlation coefficient (r) Statistical significance $p < 0.05$.

In the current study, anthropometric, biochemical, inflammatory and micronutrient statuses in apparently healthy adult volunteers (n = 200) were examined in this cross-sectional study. Mostly, participants were overweight and the value of the average BMI revealed higher cardiometabolic risk. Biochemically, dys regulation of glycaemic metabolism and vitamin D deficiency in participants could be reported and the hs-CRP level was quite higher. However, BMI was positively and significantly correlated with hs-CRP and HbA1c, whereas vitamin D showed negative significant relationship with BMI. Insignificant weaker relationship was established between BMI and serum magnesium and zinc, respectively. Higher level of triglyceride along with rise of hs-CRP and reduced vitamin D level could indicate that metabolic syndrome with common links could be observed which is related to dyslipidaemia as well as inflammation which implies relationship between adiposity with dysfunction of glycaemic regulation and chronic inflammation as well as micronutrient deficiency. Hence, examination of overall anthropometric, Biochemical, Inflammatory, Lipid and micronutrient profiles should play vital role in early risk prediction of participants with increased cardiometabolic diseases risk in order.

4. DISCUSSION

This cross-sectional study was carried out to explore the relationship of anthropometric characters, serum micronutrients, inflammatory parameters, and cardiometabolic biomarkers among apparently healthy working individuals. None of the participants suffered from clinically recognised metabolic disease at the time of enrolment; however, variability existed with respect to body composition, glycaemic profile, inflammatory status, and levels of micronutrients [19]. It indicates the development of metabolic disturbance well before clinically diagnosed disease appearance hence it can be concluded that health evaluation of apparently healthy persons in a comprehensive way in workplace provides an important way to screen these patients and apply effective prevention strategies [20].

So combined assessment of anthropometry, biochemical parameters, inflammatory and nutrition markers in workplace seems to be a useful tool to identify the population for early intervention. The population under investigation mostly includes middle aged workers with the mean value of BMI belonging to overweight category thus the problem of overweight is widely seen among apparently healthy workplace workers as well [21]. This problem has become the major contributor to non-communicable diseases, globally. We found significant positive correlations of BMI with Haemoglobin, Iron, hs-CRP and HbA1c and significant negative correlation with serum vitamin D concentration, indicating that with increase in adiposity

metabolic and Nutritional, inflammatory alternations appear before apparent metabolic disease presentation inapparently healthy subjects [22]. Several occupations and population-based studies also support the role of obesity in metabolic disorder. Obesity has already been considered as the main factor that influences various processes leading to insulin resistance, inflammation, dyslipidaemia, metabolic syndrome and CVD. Therefore, BMI remains the simplest and inexpensive screening test and an efficient tool in the occupational settings for assessing risk of cardiovascular disease [23,24].

Now obesity is seen as a complex chronic metabolic disorder not as an accumulation of adipose tissue. Adipose tissue secretes diverse group of mediators such as adipokines, cytokines, chemokines and other molecules which are responsible for regulation of systemic metabolism. Macrophage infiltration to adipose tissue increases with increased adiposity that leads to enhanced production of tumour necrosis factor-alpha, interleukin-6, leptin, resisting etc and decreased level of protective adiponectin that initiate chronic low-grade inflammation, oxidative stress, endothelial dysfunction, impaired insulin signalling and mitochondrial dysfunction which contributes to development of type 2 diabetes, hypertension, metabolic syndrome, NAFLD and atherosclerotic CVD [25]. Thus, positive association between BMI and hs-CRP as found in this study seems logical in perspective of obesity induced inflammation as early event in process of Cardiometabolic disease development. Visceral adipocyte expansion releases pro-inflammatory cytokines (primarily IL-6), which stimulate hepatic hepcidin production [26]. Elevated hepcidin degrades ferroprotein, trapping iron in storage cells. This creates functional iron restriction-limiting iron availability for erythropoiesis and lowering haemoglobin (Hb) levels without necessarily requiring absolute body iron depletion. Reduced Hb decreases oxygen-carrying capacity, while intracellular iron restriction compromises iron-dependent mitochondrial enzymes [27]. This cellular hypoxia and altered mitochondrial function act as potential contributors to metabolic stress, rather than a direct, singular cause of insulin resistance 35 Chronic low-grade inflammation directly interferes with insulin signaling, facilitating higher blood glucose levels that coexist alongside nutritional and haematological abnormalities [28]. Notably, iron deficiency and altered erythrocyte turnover can independently alter HbA1c levels separate from actual glucose exposure, making this a crucial confounding variable to address when evaluating glycaemic status [29].

We have evidence of alternated glucose metabolism even among apparently healthy employees as few of them were with increased values of fasting glucose and HbA1c that falls within normal range of blood glucose but they would fall into higher categories as if they were the subject of investigation as the general population (i.e. Non employees). Thus, it indicates that impaired glucose homeostasis may develop earlier even before clinical symptoms manifest, and positive correlation between BMI and HbA1c suggesting the contribution of adiposity to the deteriorating long-term glycaemic control [30]. This relation may be related to the phenomenon of insulin resistance where increase in adiposity can contribute to decrease insulin sensitivity by means of chronic inflammation, ectopic lipid accumulation, mitochondrial dysfunction, oxidative stress and impaired adipokine production. This leads to an increased insulin secretion requirement by pan created-cells in order to compensate impaired insulin sensitivity and maintain glucose homeostasis. If it continues then insulin sensitivity decreases leading to impairment of -cell function resulting in the loss of glucose tolerance and progression to type 2 DM. HbA1c measure long term glucose control for past 2-3 months, hence less sensitive to short-term fluctuations than fasting plasma glucose, it's reliable for diagnosis of diabetes and for evaluating glycemic control. So, assessment of HbA1c in apparently healthy workforce is suggested [31]. Early intervention by lifestyle modification like diet counselling, increased physical activity, weight management and behaviour therapy can limit future risk of both Diabetes and CVD.Our study also found that hypovitaminosis D was widespread in the apparently healthy subjects studied (41.1% had vitamin D deficiency). Low level of vitamin D was found to be significantly negatively associated with BMI indicating that higher level of obesity was linked with increased prevalence of hypovitaminosis D which has already been documented in several epidemiological studies in a number of countries and obesity and vitamin D deficiency are closely related factors in the etiology and progression of cardiometabolic disease [32].

This relation can be due to a decrease in absorption of orally intake Vitamin D due to increased visceral fat mass, decrease of exposure to sunlight and increased vitamin D metabolic inactivation. Since vitamin D is fat-soluble vitamin, it tends to get stored in adipose tissue, and as the amount of adipose tissue increases, a higher fraction of vitamin D becomes sequestered in fat tissue, which makes it unavailable to be circulating in the blood stream and thereby the deficiency develops. Vitamin D has effects other than Ca homeostasis and skeletal metabolism including glucose metabolism, insulin sensitivity, lipid homeostasis, and inflammatory and immune functions [33]. Pancreatic -cells, adipocytes, endothelial cells, skeletal muscles and immune

cells are targets of vitamin D receptors and the present data are supported by associations of vitamin D with insulin resistance, endothelial function, oxidative stress and inflammatory processes causing risk to metabolic syndrome and type 2 diabetes mellitus (T2DM) as well as cardiovascular diseases (CVD) where vitamin D has shown to be a predictor. Therefore, the present inverse relation between BMI and vitamin D may lead to the conclusion that low levels of vitamin D can be an early nutritional index in apparently healthy workers predisposed to cardiometabolic dysfunction [34]. Health status monitoring through plasma concentrations of vitamin D may improve early prediction of workers needed nutrition and lifestyle interventions especially in employment locations with prolonged staying indoors and deprived of sunlight, thus contributing to prevention of metabolic diseases. Because of the role in the maintenance of metabolic homeostasis, iron, magnesium, zinc, and vitamin B12 together with calcium have been determined. The plasma values are highly variable due to diet, lifestyle and health status. Mg is important for glucose transport, ATP metabolism, mitochondrial performance and insulin signaling and Zinc for-cell functioning, insulin synthesis and secretion, oxidant stress and immunity control; the obtained relation between plasma Mg and Zn and BMI provide clues as regards its possible role in the metabolic alteration of obese individuals. Serum Fe, Ca and vit B12 were negatively associated with BMI, HbA1c and hs-CRP respectively, suggesting they are somehow related to the parameters being tested in this work even if, due to statistical limitations of the present analysis, their potential implication is not guaranteed [35]. Positive association between BMI and hs-CRP means that as adipose tissue increases inflammation rises as a whole; hypertriglyceridemia induces adipocyte lipolysis, oxidative stress and inflammatory cytokines release (IL-6, TNF-), provoking increase ofhs-CRP production by liver. Reduced amount of vitamin D would intensify lipid alterations, insulin resistance and inflammatory profile. Association between high triglycerides, high hs-CRP levels and reduced amounts of vitamin D is likely contributing to weight increase, insulin resistance, diabetes mellitus, cardiovascular events and other inflammatory diseases; chronic hyperglycaemia, assessed as increased HbA1c, in association with raised inflammation-marker like hs-CRP underlines relationship between these two parameters [36]. These results have significant implications as they suggest how we can diagnose people within the workforce who are already displaying multiple risk factors, but without being aware of it from the subjective point of view. Even though patients may apparently seem healthy, they might actually have already gained adiposity, deteriorated glucose control, sub-clinical inflammation as evaluated by raised hs-CRP level, reduced amounts of vitamin D, changes in selected micronutrient plasma levels compared with referent ranges. Accordingly, the combination of anthropometry assessment (measurements of waist/height, hips/waist and BMI), estimation of fasting blood glucose and glycosylated haemoglobin (HbA1c), determination of inflammatory markers-CRP, vitamin D and selected micronutrients (e.g. Mg) will facilitate early intervention in order to improve employee's profile through adequate nutrition advising, physical activity, weight management and appropriate supplementation [37]. Main study strengths consist in measuring concurrently anthropometric, glycemia, inflammation parameters, hormonal and haematological and few micronutrients' values in an adequately large number of working individuals but they are also limitations by definition since no cause-consequence relations can be concluded from a cross-sectional evaluation; moreover, as results derive from just one cohort, it seems difficult to generalize it [38]. Dietary patterns, physical activity, hours of sleep, smoking, alcohol consumption, exposure to sun, medications history, family history were collected only subjectively, so this could influence in many respects of assessment and conclusion-draw; future prospective, multicentre studies with specific lifestyle interventions as vitamin D or selected micronutrient supplemented could provide clearer indications as regards these findings. Evaluation of other marker of inflammatory processes, different hormones or nutrients and testing targeted intervention strategies designed at reducing cardiovascular risk could be very helpful [39,40].

5. CONCLUSION

This cross-sectional study comprehensively evaluated the anthropometric parameters, glycaemic biomarkers, inflammatory markers, hormonal profile, serum micronutrients and haematological parameters among ostensibly healthy working adults. Despite not having diagnosed cardiometabolic disease, the results highlighted substantial between-person variability in metabolic and nutritional health and indicate that subclinical physiological changes may have already occurred prior to overt clinical presentation of disease. This highlights the need to identify subclinical metabolic dysfunction at the earliest possible stage of development, where intervention is likely to be most effective. The study also revealed that relying solely on routine clinical assessment is likely to underestimate the prevalence and impact of emerging cardiometabolic risk in occupational populations. The study's most significant observation was the high prevalence of overweight and obesity among the subjects, and that BMI was significantly correlated with several clinically relevant metabolic parameters. Increasing BMI was associated with higher HbA1c, hs-CRP, and lower vitamin D concentration. This confirms the intimate interrelation between adiposity,

impaired glucose metabolism, chronic low-grade systemic inflammation, and nutrition imbalance. Our finding is in line with the paradigm that obesity acts as a metabolically active disease process capable of initiating inflammatory and metabolic events long before the appearance of clinically identifiable type 2 diabetes mellitus and cardiovascular disease. Another notable finding of the study was that a significant proportion of seemingly healthy employees demonstrated signs of impaired glucose homeostasis. The relatively high mean fasting glucose and HbA1c among these individuals reflect the possible early, silent changes in glucose tolerance in early metabolic dysfunction. Also, increased BMI showed significant relation to HbA1c and suggests that increase in body weight may adversely affect the long-term control of glucose and insulin resistance. It may be worthwhile to include routine measurement of fasting glucose and HbA1c in the workplace occupational screening of employees to identify those with impaired glucose metabolism who would benefit from early lifestyle modification before the irreversible development of complications. Additionally, high prevalence of vitamin D deficiency was observed, accompanied by a wide spectrum of vitamin B12, iron, zinc, magnesium, and calcium serum concentrations. Among these micronutrients, vitamin D had the strongest correlation with obesity, thus indicating vitamin D deficiency might be an early marker of metabolic dysfunction in healthy working adults. While remaining micronutrients only weakly or not significantly associated with any cardiometabolic markers, the variation in their serum concentration suggests the impact of nutrient intake on metabolic health. This observation supports the growing consensus on the benefits of complementary nutrient assessments in the routine Biochemical investigations used for preventive health care and occupational health screenings. Especially, workers living a sedentary life with less sun exposure due to indoor workplaces might represent an increased risk for deficiency. Persistent hyperglycaemia and adiposity create a feedback loop that elevates systemic inflammatory markers like hs-CRP. This combined metabolic and inflammatory dysregulation promotes endothelial dysfunction and vascular remodelling, signaling increased long-term cardiometabolic risk rather than baseline clinical cardiovascular disease. Iron status and haemoglobin are important components of cardiovascular health. Evidence from observational studies shows that iron depletion and low haemoglobin are associated with coronary artery disease and poorer cardiovascular outcomes. In patients with heart failure, iron deficiency is common and is associated with impaired functional capacity, hospitalization and adverse outcomes. Importantly, cardiovascular studies suggest that iron deficiency may have clinical consequences even in the absence of overt anaemia, while low haemoglobin may further compromise oxygen delivery and cardiac function. Therefore, altered iron availability and reduced haemoglobin may represent important nutritional and haematological components of cardiometabolic risk. Positive association between BMI and hs-CRP confirmed the presence of chronic low-grade inflammation associated with increased adiposity. Because hs-CRP is known as a reliable marker for systemic low-grade inflammation related to obesity and an independent predictor for the risk of cardiovascular disease, routine measurement of hs-CRP may improve the detection of individuals with metabolic syndrome or other related outcomes. Contrary to our expectation, there was no statistically significant correlation between BMI in this study. In conclusion, this study established that seemingly healthy working adults carry an excess load of numerous cardiometabolic risk factors including obesity, impaired glycaemic regulation, systemic low-grade inflammation, vitamin D deficiency and varied status of major micronutrients. Associations of increased BMI withHbA1c, hs-CRP and lower vitamin D concentration reveal a complex relationship among adiposity, inflammation, nutrition and metabolic health, which can develop in subclinical stage of metabolic dysfunction. This emphasizes the need to shift towards comprehensive occupational screening including measurement of anthropometric, glycaemic, inflammatory and micronutrient status besides regular clinical examination, so that those at high risk can be identified early and can be provided appropriate lifestyle and nutritional intervention to reduce their later burden of type 2 diabetes mellitus and cardiovascular diseases. Collectively, these findings provide convincing evidence for the adoption of preventive, evidence-based occupational healthcare policies designed to optimize healthy workplaces and contribute to improved public health.

STATEMENT AND DECLARATION

ETHICS APPROVAL

Ethical clearance was obtained from the Institutional Human Ethics Committee of PSG Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India (Approval No. PSG/IHEC/2026/Appr/FB/021).

CONSENT TO PARTICIPATE

Written consent was obtained from the patient to participate in the study anonymously.

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COMPETING INTERESTS

The authors declare no competing interests.

Availability of data

Data available on request.

FINANCIAL INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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