ORIGINAL RESEARCH

Cardiovascular disease risk factors and the ten-year Cardiovascular disease risk among employees of a public university in Blantyre, Malawi

Milca Mzunga[1], Gomezgani Msiska[1], Cecilia M. Phiri[2], Victoria Mukhula[2,3], Angel J. Mtonga[4], Dikani Salema[3], George Chirambo[3,5], Asante Makuta[6], Fanuel Lampiao[5], Adamson Muula[7], Mwapatsa Mipando[3,5], Kondwani G.H. Katundu[2,3,6*]

1. Department of Medical Laboratory Sciences, School of Life Sciences and Allied Health Professions, Kamuzu University of Health Sciences, Blantyre, Malawi
2. Malawi-Liverpool Wellcome Programme Clinical Research Programme, Blantyre, Malawi
3. Blantyre to Blantyre Research Facility, Kamuzu University of Health Sciences, Blantyre, Malawi
4. Kamuzu University of Health Sciences, Blantyre, Malawi
5. Department of Biomedical Sciences, School of Life Sciences and Allied Health Professions, Kamuzu University of Health Sciences, Blantyre, Malawi
6. Nutrition and Dietetics Department, School of Global and Public Health, Kamuzu University of Health Sciences, Blantyre, Malawi
7. Department of Public Health, School of Global and Public Health, Kamuzu University of Health Sciences, Blantyre, Malawi

***Corresponding Author: Kondwani G.H. Katundu**; E-mail:kkatundu@kuhes.ac.mw

Abstract

Background

Atherosclerotic Cardiovascular diseases (ASCVD) are the leading cause of death globally and disproportionately affect developing countries such as Malawi. Screening and management of significant ASCVD risk factors is essential. In Malawi, the prevalence of ASCVD risk factors among university staff, an economically important population, has not been well reported. This study investigated the prevalence of risk factors for ASCVDs and the ASCVD risk among employees of the Kamuzu University of Health Sciences, in Blantyre, Malawi.

Methods

This was a cross-sectional study conducted in 2022 with 105 participants. Sociodemographic data, medical history, and anthropometric measurements were recorded. Blood samples were analyzed for HbA1C and serum lipids. Associated risk factors for ASCVD were assessed. The 10-year risk for ASCVD was calculated based on the Framingham Risk Table to predict 10-year cardiovascular outcomes. Statistical analysis included descriptive statistics and multivariate logistic regression to evaluate associations while adjusting for potential confounders.

Results

Overweight and obesity were prevalent in 78% of the participants, while 43% and 42% of the participants had blood pressure readings in the pre-hypertension and hypertension categories, respectively. A proportion of 21% and 8% of the participants had HbA1c readings in the category of pre-diabetes and diabetes, respectively. Dyslipidaemia was detected in 72% of the participants. Regarding ASCVD risk, 58% had intermediate risk, while 13% had high risk. Being overweight or obese and age over 30 years predicted intermediate to high ASCVD risk (AOR 1.24 (95% CI 1.04 – 1.49) $p=0.02$, and (AOR 1.51 (95% CI 1.16 – 1.97) $p < 0.01$, 0.0001 respectively.

Conclusion

This study reveals a high prevalence of ASCVD risk factors among KUHeS employees, with two-thirds of the population having an intermediate or high 10-year ASCVD risk. Routine screening and tailored management strategies are essential to reduce the ASCVD risk and burden in this population.

Keywords: Dyslipidemia, Diabetes Mellitus, Hypertension, Overweight and Obesity, Atherosclerotic Cardiovascular Disease, KUHeS, Public University, Malawi

Introduction

Atherosclerotic Cardiovascular diseases (ASCVDs) are the leading cause of premature death and disproportionally significantly affect Sub-Saharan Africa, Malawi inclusive[1-3]. A 2017 STEPwise approach to surveillance (STEPS) survey for chronic non-communicable diseases (NCDs) reported that NCDs in Malawi, including ASCVDs, are responsible for 32.0% of all deaths[3]. ASCVDs alone have a prevalence of 8.9% and account for 12% of all adult deaths in Malawi[4]. Furthermore, Malawi has a high age-standardized mortality rate (ASMR) of 250-325 per 100,000 individuals for ASCVDs[4].

ASCVDs and the associated burden can be curbed, as 70% of all ASCVDs are attributed to modifiable risk factors which include dyslipidemia, high blood pressure, overweight and obesity, diabetes mellitus, physical inactivity, alcohol abuse, and tobacco smoking[5,6].

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Primary and secondary prevention of ASCVD is essential in all people at a higher risk of cardiovascular diseases. In Low-Income Countries (LICs) such as Malawi, ASCVDs disproportionately affect the socioeconomically advantaged population[7], contributing significantly to the country's economy. The growth of any economy is tied to the government's taxation structure[8]. Like many other developing nations, Malawi heavily relies on tax revenues and donor aid to finance its budgets[9]. Taxes, including Pay-As-You-Earn (PAYE), are significant in generating revenue to support government spending, with PAYE contributing the second-highest amount to the gross revenue after Value Added Tax (VAT)[10]. A healthy human capital structure is a crucial determinant of a country's economy (11). Individuals in this structure are vital to the economy through their productivity[10]. However, ASCVDs and their associated high mortality rates, particularly premature deaths and prolonged morbidity, result in decreased productivity and affiliated low per capita income growth rate[12,13].

Given the relatively small proportion of taxpayers, coupled with a significant proportion of the rural population, ASCVDs pose a substantial risk to the economy of Malawi. Therefore, addressing ASCVD risk is imperative for sustaining Malawi's economic growth and ensuring the well-being of its workforce. Assessing cardiovascular risk, discussing a heart-healthy lifestyle, and managing risk factors are essential for reducing the burden of ASCVDs[14,15]. Such an approach ensures timely interventions to prevent premature deaths and years lost due to morbidity due to ASCVDs and associated economic loss[3].

Malawi lacks data on the prevalence of ASCVD risk and risk factors among the working class, such as public university employees, a population group at risk, given their socioeconomic status and risk for a sedentary lifestyle due to the nature of their work[6]. These employees form part of a population group of individuals who significantly contribute to the economy[1,16]. This cross-sectional study aimed to determine the ASCVD risk and the prevalence of ASCVD risk factors among employees at Kamuzu University of Health Sciences (KUHeS) in Blantyre, Malawi.

Methods

Study design and setting

This cross-sectional study was conducted at the Blantyre Campus of the Kamuzu University of Health Sciences (KUHeS), Malawi. KUHeS is a new public university formed in 2019 after the delinking and merger of the former College of Medicine and the Kamuzu College of Nursing at the University of Malawi. The study participants were enrolled between August and September 2022.

Study population, sampling, Inclusion and exclusion criteria

The study population was KUHeS employees aged 18 and above at the Blantyre campus. KUHeS has two campuses, Blantyre and Lilongwe. The total number of staff from both campuses is 972, distributed as follows: 350 academic staff, 347 administrative staff, and 225 research project staff. For this study, only academic and administrative staff from Blantyre were considered resulting in a sampling frame of 463 eligible participants, 49% of which were academic staff. A systematic sampling method was employed to recruit the participants. The sampling frame was constructed using the official KUHeS employee directory, which lists all eligible staff alphabetically. To ensure randomness, the first participant was selected using a random number generator, after which every second individual on the list was included until the target sample size of 105 participants was reached. This approach ensured that each eligible individual had an equal probability of being selected, maintaining the sample's representativeness.

We excluded participants who reported being pregnant, those who reported an acute disease condition, and those who gave incomplete information as per the study. The sample size for the study was 105 participants, and the sample size was reached at to detect a prevalence of at least 40% cardiovascular risk factors.

Data Collection

Data were collected and documented using an interviewer-guided questionnaire. The questionnaire used for data collection was adapted from validated tools and was pretested on a small subset of employees at a similar institution to ensure clarity, reliability, and relevance of the questions. Collected data included social-demographic data such as age, sex, gender, the highest level of education, occupation, the position they hold at the university, smoking and alcohol intake in the past 3 months, and medical histories such as previous heart attack, stroke, and diabetes. Blood pressure measurements were performed and recorded. Anthropometric measurements, including weight and height, were performed, from which the body mass index (BMI) was calculated and classified according to the National Institute of Health and the World Health Organization (WHO) classification[17]. Waist and hip circumferences were also measured, from which the waist-hip ratio was calculated. Anthropometric measurements and blood pressure were standardized using calibrated equipment, and all measurements were taken by trained personnel following WHO protocols to minimize measurement error.

Whole venous blood samples were collected, and glycated haemoglobin (HbA1c) tests were performed using HbA1c EZ 2.0 (Wuxi BioHermes Biomedical Technology Co., Ltd, China). The remaining blood samples were collected in a vacutainer tube without an anticoagulant and stored on ice. Samples were then centrifuged within three hours and stored in -80 degrees Celsius freezers until lipogram analysis. The serum samples were analysed for LDL-C, TG, TC, and HDL using a Beckman AU480 Chemistry analyser at the Blantyre-Blantyre Research Facility at the Kamuzu University of Health Sciences.

Definitions

Dyslipidaemia was defined as any of the following lipid abnormalities: TC $\geq$ 4 mml /L, TG $\geq$ 1.7 mml/L, LDL-C $\geq$ 3 mm/L, and HDL-C >1 mml/L. All the dyslipidaemia patterns were classified except total cholesterol, and the presence of a single abnormal lipid parameter (TC, TG, HDL-C, or LDL-C) was classified as isolated dyslipidaemia[2], the presence of two abnormal lipid parameters (elevated TG, low HDL-C, or elevated LDL-C) as combined dyslipidaemia; and the abnormality of three indices (TG, HDL-C, and LDL-C) as mixed dyslipidaemia. No dyslipidaemia was classified as either TG/HDL/LDL.

Glycated haemoglobin (HbA1c) of < 5.7% was considered normal, 5.7-6.5% was prediabetes, and >6.5% as diabetes.

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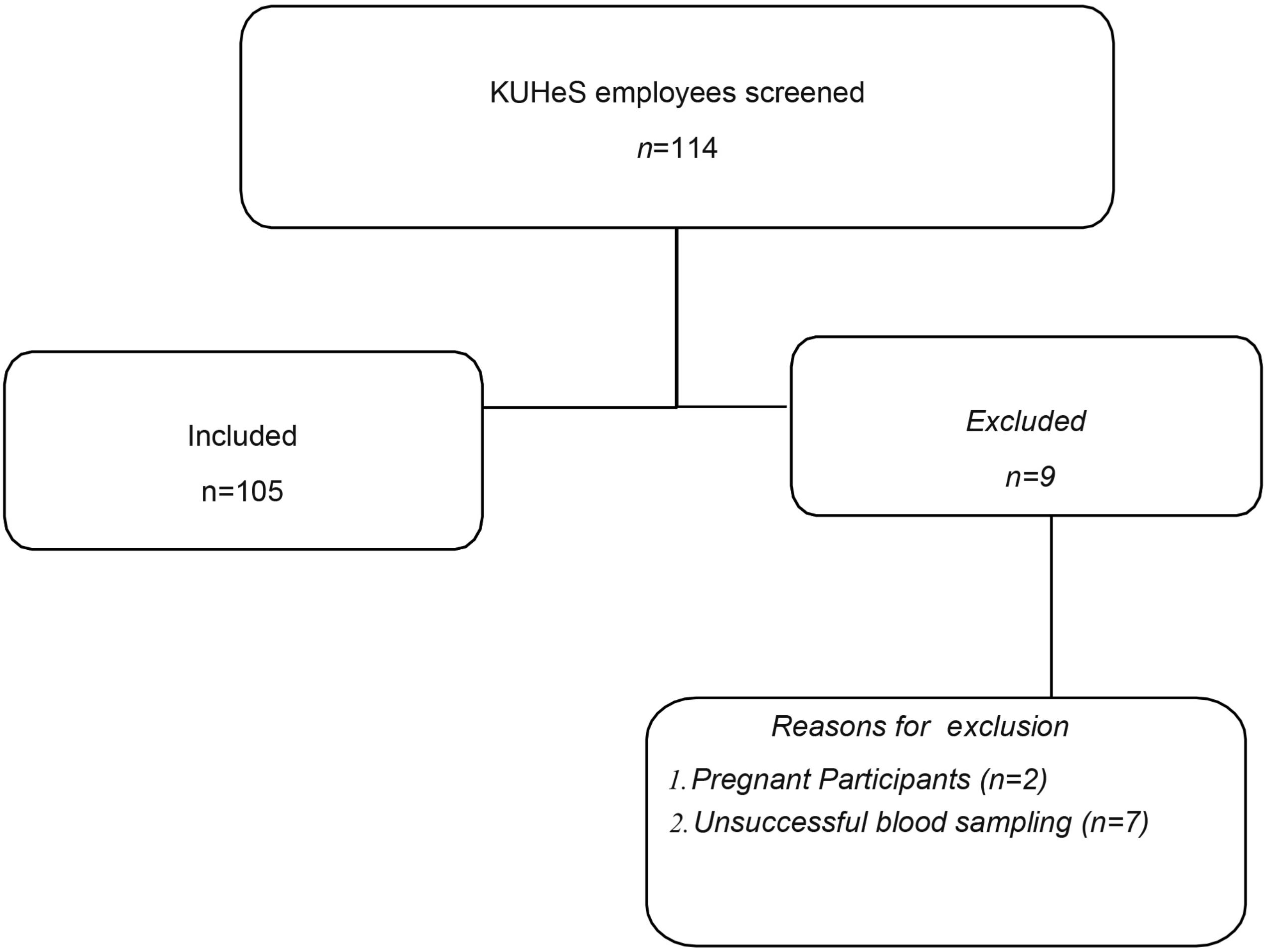

Figure 1: Strobe diagram illustrating the number of participants screened, qualified for inclusion, and the reasons for exclusion in the study. n = Number

Blood pressure categories were defined as ≤120/≤80, as normal; 120/139/80-89, as pre-hypertension; and ≥139/≥90, as hypertension. Body Mass Index (BMI) was defined as the following: <18.5-24.9, as normal; 25.0-29.9, as overweight; and 30.0-60.0 as obese.

Regular exercise was defined as engaging in at least 150 minutes of moderate-intensity aerobic physical activity, or 75 minutes of vigorous-intensity aerobic physical activity, or an equivalent combination of both, spread throughout the week.

The 10-year risk for ASCVD was categorized based on the Framingham Risk Score (FRS) as, Low Risk: less than 10% chance of developing cardiovascular disease in the next 10 years; Intermediate Risk: 10% to 20% chance; High Risk: greater than 20% chance. The FRS incorporates factors such as age, sex, blood pressure, cholesterol levels, diabetes, and smoking status and is extensively used for predicting the 10-year risk of ASCVD in the general population (18). The tool has been validated across different populations, although local adaptations are often necessary for accurate predictions[18].

Statistical analysis

Data were entered into Microsoft Excel, and statistical analysis was performed using Stata17 software (StataCorp, USA). Descriptive statistics were presented as means or medians for continuous variables like age and lipid levels, and as proportions for categorical variables. The prevalence of different conditions was calculated as a proportion among participants. The Chi-squared or Fisher's exact test was used to compare categorical data, while t-tests analyzed mean differences. Logistic regression assessed the cardiovascular risk across different age groups, job categories and BMI categories, with multivariate analysis controlling for confounders. A p-value < 0.05 was deemed significant.

Results

Characteristics of the study participants

Figure 1. summarises the number of participants screened for the study and the reasons for exclusion. A total of 114 participants were screened for inclusion criteria, and of these, 105 were enrolled, while nine individuals were excluded from the study either because they were pregnant or failed to provide a venous blood sample. Table 1 summarises the characteristics of the study participants, with virtually an equal distribution between males and females. The median age for the study participants was 37.0 years old (IQR 28.0 – 46.0 years old). Of the study participants, 76.0% were over the age of 30 and none of the participants were above the age of 60 years. Most of the study participants (94.3%) reported having no previous history of diabetes while 4.7% had type 1 diabetes and 0.9% had type 2 diabetes. The results also depicted that 15.2% of the study participants were known hypertensive. Upon blood pressure measurement, 86% of the participants were either categorized as prehypertensive or hypertensive.

According to BMI assessment, 78% of the participants were either overweight and obese with a median BMI of 28.1 kg/m^2 (IQR 25.6 – 32.5 kg/m^2).

Table 1: Characteristics of the study participants

Characteristic	Category	N (%)
Sex	Male	53 (50.5)
	Female	52 (49.5)
Age, years [Median (IQR)]		37.0 (28.0 – 46.0)
	<30 years	29 (27.7)
	≥ 30 years	76 (72.3)
Occupation	Supporting Stuff	72 (68.6)
	Academic	33 (31.4)
Education	Below Degree	27 (25.7)
	Bachelor's Degree	60 (57.1)
	Master's Degree	14 (13.3)
	PhD	4 (3.8)
Alcohol Consumption	Yes	26 (27.6)
	No	76 (72.4)
Regular Exercise	Yes	66 (62.9)
	No	39 (37.1)
Previous Cardiovascular Disease	No	101 (96.2)
	Stroke	2 (1.9)
	Heart attack	2 (1.9)
Diabetes Mellitus (DM)	No DM	99 (94.3)
	Type 1	4 (3.8)
	Type 2	1 (0.9)
	Gestational DM	1 (0.9)
Hypertension	Yes	16 (15.2)
	No	89 (84.8)
BMI, Median (IQR)		28.1 (25.6 – 32.5)
	Normal (18.5-24.9)	23 (21.9)
	Overweight (25-29.9)	45 (42.9)
	Obese (30-60)	37 (35.2)
Waist Hip Ratio, Medium (IQR)		0.8 (0.8 – 0.9)
	<=0.9M/0.85F	79 (75.2)
	>=0.9M/0.85F	26 (24.8)
HbA1c, Median (IQR)		5.3 (5.0 – 5.7)
Normal	<5.7	74 (70.5)
Prediabetes	5.7-6.5	23 (21.9)
Diabetes	>6.5	8 (7.6)
Blood Pressure, mmHg		
Systolic [Median (IQR)]		130.0 (121.0 – 148.0)
Diastolic[Median, (IQR)]		84.0 (76.0 – 94.0)
	Normal	16 (15.2)
	Prehypertension	45 (42.9)
	Hypertension	44 (41.9)

Female participants had a significantly higher median BMI (29.4 kg/m^2 (IQR 26.3 – 33.6 kg/m^2)) compared to male participants (27.4 kg/m^2 (IQR 24.5 – 29.8 kg/m^2)), p-value 0.03. Based on the HbA1c measurements, 30% of the participants were categorized as either prediabetic or diabetic (Table 1).

Prevalence and patterns of dyslipidaemia in the study population

Lipid analysis showed that 71.5% of the study participants had at least one form of dyslipidaemia. Notably, the most prevalent form of dyslipidaemia was combined dyslipidaemia, followed by mixed dyslipidaemia, and the least prevalent form of lipid abnormality was isolated dyslipidaemia.

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Table 2: Distribution pattern of dyslipidaemia among the study participants (n = 105)

Dyslipidaemia type	Parameter	N (%)
No dyslipidaemia		4 (3.81)
Isolated dyslipidaemia (single lipid abnormality)		15 (14.3)
	TC	15 (14.3)
	LDL-C	0(0)
	TG	0(0)
	HDL-C	0(0)
Combined dyslipidaemia (two lipid abnormalities)		40 (38.1)
	LDL-C + TG	11(10.5)
	LDL-C + HDL-C	27(25.7)
	HDL-C + TG	1(0.95)
Mixed dyslipidaemia (> two lipid abnormalities)		
	LDL-C + TG + HDL-C	39 (37.2)

TC = total cholesterol; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; TG = triglycerides

Table 3: Association between selected variables and cardiovascular disease risk in study participants

Variable	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Sex	1.04 (0.32-3.35)	0.95	0.71 (0.11 – 4.39)	0.71
Ref: Male				
Overweight and Obesity	1.15 (1.04-1.26)	<0.01	1.24 (1.04 – 1.49)	0.02
Age> 30 years	1.41(1.17-1.71)	<0.01	1.51 (1.16 – 1.97)	<0.01
Dyslipidaemia	1.85 (0.49 – 7.01)	0.36	178 (0.25 – 12.86)	0.57

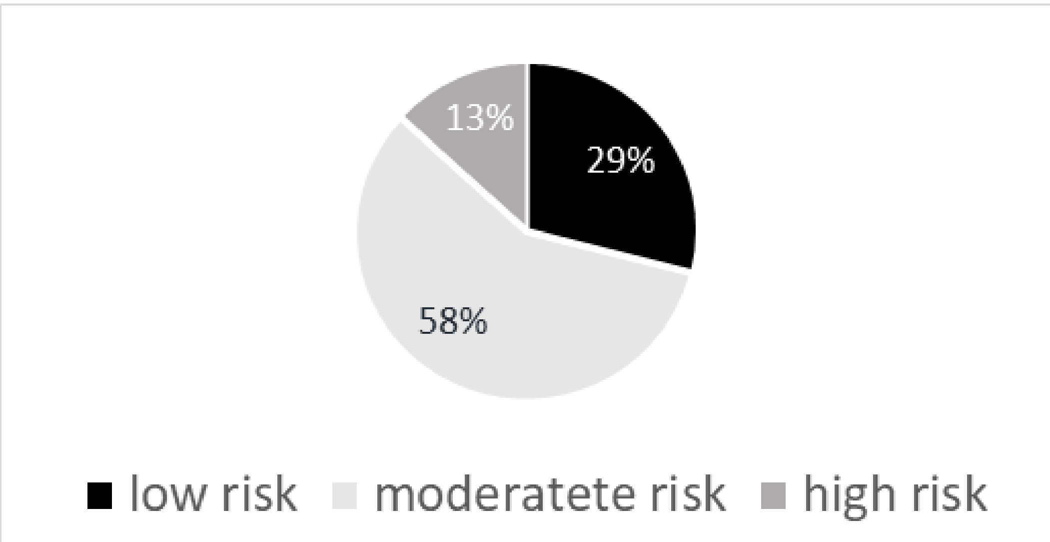

Figure 2: A pie chart illustrating 10-year atherosclerotic cardiovascular disease risk categories using the Framingham risk table. Low Risk = less than 10% chance of developing cardiovascular disease in the next 10 years; Intermediate Risk = 10% to 20% chance; High Risk = greater than 20% chance

Across all dyslipidaemia categories, elevated levels of HDL-C + LDL-C in combination were the most prevalent lipid abnormality observed in 25.7% of the study population (Table 2).

Atherosclerotic cardiovascular disease risk among the study participants

We calculated the 10-year ASCVD risk distribution in the study population using the Framingham risk table. Figure 2. illustrates the findings. Notably, 71% of the participants were either moderate or high risk and were therefore classified as having a high risk of ASCVD over the next decade.

Risk factors associated with cardiovascular diseases among the study participants

The 10-year cardiovascular disease risk was positively associated with age >30 years old and BMI. The risk was not statistically significantly associated with sex or dyslipidaemia. Upon multivariate analysis, age and BMI were maintained as independent predictors of cardiovascular diseases in the participants (Table 3).

Discussion

The present study at a public university in Southern Malawi aimed to establish the prevalence of ASCVD risk factors and the 10-year ASCVD risk among healthy employees. We observed a high prevalence of ASCVD risk factors among the study participants. The most prevalent risk factor was dyslipidemia which was virtually in all the participants, followed by overweight and obesity, pre-diabetes and diabetes and pre-hypertension and hypertension. Approximately three-quarters of the study population had moderate to high ASCVD risk. The 10-year cardiovascular disease risk was positively associated with age above 30 years and overweight or obesity. Both factors emerged as independent predictors of ASCVD risk among the study participants.

Cardiovascular diseases are currently the leading cause of death worldwide, with a disproportionate burden in developing countries, causing twice as many deaths as malaria, tuberculosis, and HIV/AIDS combined[19,20]. The ASCVD burden in developing countries is driven by increasing risk factors and a lack of reinforcement of interventions to address the risk factors[21]. If not addressed, ASCVDs may negatively affect the economic development of a country[22]. ASCVDs often affect individuals in the prime working years who significantly contribute to the country's Gross Domestic Product (GDP), resulting in the loss of productivity and taxation revenue[23]. The World Health Organization (WHO) advocates for healthier workplaces to curb ASCVD risk factors[22].

The findings of the present study are similar to other studies on the high prevalence of cardiovascular risk factors among African university staff , with significant occurrences of hypertension, obesity, dyslipidaemia, and lifestyle-related conditions[24-28]. The present findings also highlight notable differences in cardiometabolic risk factors between university employees and the general Malawian population, as reported in the recent Malawi National STEPwise Survey for Non-Communicable Diseases Risk Factors[3]. Overweight and obesity were approximately four times more prevalent among university staff (78%) compared to the national average of 18.5%, reflecting the influence of sedentary work environments and higher socioeconomic status. Similarly, the prevalence of hypertension was significantly higher in our cohort (42%) than the 16.1% observed in the general population, despite the national decline from 32.9% in 2009. The prevalence of pre-diabetes (21%) and diabetes (8%) in university employees also far exceeded the 1.4% and 5.6% reported in the national survey. Furthermore, the high prevalence of dyslipidaemia affecting the university staff, emphasizes a substantial burden of cardiovascular risk. These findings highlight the unique vulnerabilities of university employees, likely driven by occupational and lifestyle factors and point to the need for tailored workplace interventions to complement community-wide efforts in addressing non-communicable diseases in Malawi.

We found a high prevalence of overweight and obesity, including central obesity, marked by a high waist-to-hip ratio among the study participants. Obesity and particularly central obesity, significantly increases the ASCVD risk[29]. The results of this study showed that ASCVD risk increases by 15% per unit of weight gained. Obesity positively contributes to cardiovascular disease risk factors such as hypertension, dyslipidemia, and diabetes, all of which are major ASCVD risk factors[30,31]. Compared to male participants, females had a higher BMI ($p < 0.026$). These results are consistent with other findings that obesity is twice as common among females than it is among males in African countries and the Middle East[32,33]. Cultural perceptions expecting females to be less physically active as well as obesity itself, are seen as a sign of high wealth status and beauty for women in the African setting[34], and likely contributes to the disparity. Despite 60% of the participants reporting regular exercise, the obesity rates in this study suggest biased reporting, poor diet, inadequate activity, or a combination of these contributing Factors.

The WHO guidelines on physical exercise recommend at least 150 minutes of moderate-intensity physical activity per week [35]. Service sector jobs such as universities are associated with decreased physical activity[36]. It is imperative, therefore, that deliberate initiatives aimed at promoting healthy lifestyles, including a healthy diet, physical activity, and weight management, are reinforced[37]. Providing sport facilities at workplaces, especially when combined with supervised exercise programs or integrated health interventions, can significantly enhance the cardiometabolic health of employees. These facilities promote increased physical activity and lead to improvements in various health markers, making them a valuable investment for employers aiming to improve employee well-being[38,39]. KUHeS has a sports complex available. However, there are no deliberate efforts and policies, such as protected time, to encourage staff members to engage in physical activities to promote cardiometabolic health. Such efforts would go a long way in addressing cardiometabolic risk factors such as the high prevalence of pre and diabetes as indicated by the high HbA1c levels reported in this study.

Dyslipidaemia, whose prevalence was alarmingly high in this study, is an important determinant of ASCVD and facilitates atherosclerosis in ASCVD[40]. Our study revealed a high prevalence of dyslipidemia. The most common lipid abnormality among the participants was combined forms of reduced HDL-cholesterol and elevated LDL-cholesterol. Elevated LDL-cholesterol paired with low HDL-cholesterol is linked to a potentiated atherosclerotic process and an increased risk for cardiovascular disease (1). High LDL-c alone indirectly causes approximately 4.3 million deaths worldwide and it has been reported to be on an exponential increase, particularly in low and middle-income countries[1,41]. Low HDL-c is linked to a sedentary lifestyle, excess weight, and uncontrolled diabetes, characteristics which were prevalent in our study population. Low HDL-c can be corrected by lifestyle changes such as weight reduction and increased physical activity[42]. Clinical guidelines emphasize the importance of regular screening and management of dyslipidemia to achieve a lower LDL-c and consequently reduce the burden of cardiovascular risk, especially in high-risk individuals[43,44].

Ultimately, the burden of cardiovascular disease can potentially decrease the country's GDP. Developing and implementing policies to reduce the ASCVDs burden is an important approach toward economic development[11]. The high rates of moderate and high 10-year CVD risk found in our study population warrant urgent attention from employers and policymakers, emphasising the need to implement strategies to ameliorate cardiovascular risk at institutions of higher learning and related public institutions. Efforts toward ensuring healthy diets at institutional workshops and meetings, promoting adequate exercise among the employees and regular screening for lipids, blood pressure, and blood glucose should be promoted[37].

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Limitations

Our study had some limitations and, hence, should be interpreted within its scope. The cross-sectional nature does not allow a cause and effect relationship of the variables studied. No dietary data was captured from the participants, and hence, inferences were only made. Additionally, self-reported diagnoses such as HIV status were subject to report bias. Another limitation of this study is the use of the Framingham Risk Score, which has not been validated for the Malawian population, despite its use globally and in other Sub-Saharan African countries (18, 45). This may have led to a potential overestimation of cardiovascular risk, as the tool was originally developed for Western populations with different demographic and lifestyle profiles. Nonetheless, this study provides important insights on the cardiovascular risk profile among university employees, and more extensive studies across several public universities in Malawi would build on the provided evidence from this study.

Conclusions and Recommendations

The results of the present study reveal a high prevalence of ASCVD risk factors among the study population and an increased risk of developing an ASCVD in the next decade. This study highlights the need for proactive approaches to reduce ASCVD risk factors and the ASCVD risk among university employees. Management of institutions and companies such as universities should invest in lifestyle modification programs that promote cardiometabolic health and reduce the risk of ASCVD. Routine annual screening for hypertension, diabetes and dyslipidaemia should be reinforced in individuals age above 30 years.

List of abbreviations

BMI: Body mass index; CI: Confidence interval; COMREC: College of Medicine Research Ethics Committee; ASCVD: Cardiovascular diseases; DM: Diabetes mellitus; HbA1c: Glycated hemoglobin; HDL-C: High-density lipoprotein cholesterol; HTN: Hypertension; IQR: Interquartile range; LDL-C: Low-density lipoprotein cholesterol; OR: Odds ratio; T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; GD: Gestation Diabetes; TG: Triglyceride; VLDL: Very low-density lipoprotein; WHO: World Health Organization; UI: Uncertainty Interval; ASMR: Age Standardized Mortality Rate; KUHeS: Kamuzu University of Health Sciences; ACEPHEM: Africa Centre of Excellence in Public Health and Herbal Medicine; QECH: Queen Elizabeth Central Hospital; IHD: Ischemic Heart Disease; NCDs: Non Communicable Diseases; SSA: Sub-Saharan Africa.

Declarations

Ethics approval and consent to participate

The study was approved by the College of Medicine Research and Ethics Committee (COMREC) before the commencement of data collection (U.11/21/3532). Permission to conduct the study on the institutions was provided by the Head of Department (HoD) of the KUHeS Medical Laboratory Sciences. Written informed consent was sought from the participants for participation in the study. To ensure privacy and confidentiality, all the participants were given codes and their data was processed using a coding system identified by Investigators at the point of sample collection. Only the Investigators had access to the identification key. The de-identified records were stored on a password-protected computer. Hard-copy records were stored in a locked filing cabinet.

Competing interest

The authors declare that they have no competing interests

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Author contribution

All authors made significant contributions to the conceptualization and the study design. MM, GM and CMP and KGHK were the primary authors of the manuscript. KGHK, MM, GC, VM, and DS were involved in executing the study. MM, DS, and GM were involved in laboratory analysis. KGHK, CMP, VM, MM, AJM, FL, AM, GM and GC contributed to the interpretation of the study results and critically reviewed the manuscript. All authors read and approved the final manuscript.

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