Sudan University of Science and Technology

College of Graduate Studies

The Relationship between Cytomegalovirus Infection and Cardiovascular Diseases in Patients attending Sudan Heart Centre

العلاقة بين عدوى الفيروس المضخم للخلايا وأمراض القلب والأوعية الدموية في المرضى المترددين على مركز السودان للقلب

A dissertation submitted in partial fulfillment for the requirements of M.Sc. degree in Medical Laboratory Sciences (Microbiology)

By :

Leena Ahmed Khairi Haroun

(B.Sc. honor Medical Laboratory Science, University of Khartoum ,(2016))

Supervised by:

Dr. Mohammed Nafi hammad

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الآيــة

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

﴿ ٱللَّهُ نُورُ ٱلسَّمَٰوَٰتِ وَٱلۡأَرۡضِۚ مَثَلُ نُورِهِۦ كَمِشۡكَوٰةٖ فِيهَا مِصۡبَاحٌۖ ٱلۡمِصۡبَاحُ فِي زُجَاجَةٍۖ ٱلزُّجَاجَةُ كَأَنَّهَا كَوۡكَبٞ دُرِّيّٞ يُوقَدُ مِن شَجَرَةٖ مُّبَٰرَكَةٖ زَيۡتُونَةٖ لَّا شَرۡقِيَّةٖ وَلَا غَرۡبِيَّةٖ يَكَادُ زَيۡتُهَا يُضِيٓءُ وَلَوۡ لَمۡ تَمۡسَسۡهُ نَارٞۚ نُّورٌ عَلَىٰ نُورٖۗ يَهۡدِي ٱللَّهُ لِنُورِهِۦ مَن يَشَآءُۚ وَيَضۡرِبُ ٱللَّهُ ٱلۡأَمۡثَٰلَ لِلنَّاسِۗ وَٱللَّهُ بِكُلِّ شَيۡءٍ عَلِيمُۢ ٣٥ ﴾

صدق الله العظيم

سورة النور: الآية (٣٥)

DEDICATION

This study is wholeheartedly dedicated to my beloved parents, who have been my source of inspiration and gave me strength when I thought of giving up, and who continually provide their moral, spiritual, emotional, and financial support.

To my husband, brother, sisters, friends, and classmates who shared their words of advice and encouragement to finish this study.

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First of all, thanks to Allah and his messenger our prophet Mohammed all peace from Allah upon him.

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ABSTRACT

Cardiovascular disease is a leading cause of death worldwide. Infectious etiology is also suspected to be a significant risk factor in these cases. Once infected, the person carries the virus for life. 80% of adults show CMV-specific antibodies in their serum indicating the high prevalence of CMV infection.

The Objective is to detect association between cytomegalovirus lnfection and cardiovascular diseases in patients attending sudan heart centre .This study was a case-control study conducted at sudan heart center, khartoum, 60 heart disease Patients as a case group and 30 healthy subjects as a control group with matched age and gender. ELISA was used to detect CMV IgG in the participant's sera.

A total of 90 individuals were participated in this study and divided into two groups; 60 (66.7%) were cases group, and 30 (33.3%) were control group with matched age and gender, regarding CVD patients, 35(58.3%) were males, and 25 (41.7%) were females in the case group. According to age groups; the age group <40 years had 22 (36.7%) patients, the age group 40-55 years had 13 (21.7%) patients, age group >55 years had 25 (41.7%) cases. Most of the patients presented with CAD 15 (25%), AF 10 (16.7%), HF 9 (15%), increase in heart palpitations 8(13%), VHD 7(11.7%), IHD 6(10%), MVR 3(5%), DCM 2(3.3%). According to the duration of diseases < 2 years had 17(28.3%), 2-3 years had 17(28.3%), and>3 years had 26(43.3%). The ELISA results of CMV revealed that there were 14 (46.7%) controls were positive for IgG, while 49 (81.7%) cases were positive for IgG from a total of 60 cases, and 30 control. There was a significant association between CMV infection and cardiovascular disease control with a *P. value* (0.001),Relative risk (5.091) .

This study shows high seroprevalence of CMV in cardiovascular patients. There was a significant association between CMV and cardiovascular diseases, thus previous infection with CMV can be a risk factor for cardiovascular diseases.

المستخلص

أمراض القلب والأوعية الدموية هي سبب رئيسي للوفاة حول العالم. يشتبه أن بعض الفيروسات قد تكون عامل خطر كبير في هذه الحالات. بمجرد أن يصاب الشخص يحمل الفيروس مدى الحياة، ٨٠٪ من البالغين يظهرون أجسامًا مضادة للفيروس في مصلهم مما يشير إلى ارتفاع معدل إنتشاره.

هدفت هذه الدراسة للكشف عن العلاقه بين عدوى الفيروس المضخم للخلايا وامراض القلب والاوعيه الدمويه في مرضى مركز السودان للقلب.

هذه الدراسة عبارة عن دراسة حالة ومجموعة ضابطة. أجريت في مركز السودان للقلب بالخرطوم. تم اختيار مرضى القلب عشوائياً. ٦٠ مريضاً بأمراض القلب كمجموعة حالة و٣٠ شخصاً أصحاء مماثلين لهم في العمر والجنس كمجموعة ضابطة. تم استخدام تقنية المقايسة المناعية المرتبطة بإنزيم للكشف عن الغلوبولين المناعي الخاص بالفيروس المضخم للخلايا. تم تحليل البيانات بواسطة برنامج الحزمة الاحصائية للعلوم الإجتماعية.

شارك ٩٠ شخصا في هذه الدراسة مقسمة إلى مجموعتين. كان ٦٠ (٦٦.٧٪) مرضي قلب، و٣٠ (٣٣.٣٪) كانوا مجموعة ضابطة، فيما يخص مرضي القلب ٣٥ (٥٨.٣٪) كانوا ذكورا، و ٢٥ (٤١.٧٪) من الإناث، أما فيما يتعلق بالفئات العمرية لمرضي قلب: الفئة العمرية أقل من ٤٠ سنة كان بها ٢٢ (٣٦.٧٪) مريض، الفئة العمرية ٤٠–٥٥ سنة كان بها ١٣ (٢١.٧٪) مريض، أما الفئة العمرية>٥٥ سنة كان بها ٢٥ (٤١.٧٪) مريض. فيما يتعلق بنوع مرض القلب ١٥ من المرضى مصابون بمرض القلب التاجي بنسبة٢٥٪، و ١٠ برجفان أذيني بنسبة ١٦.٧٪، و ٩بالفشل القلبي بنسبة١٥ ٪، ٨ يعانون من زيادة ضربات القلب بنسبة ١٣٪، ٧ مصابون بمرض القلب الصمامي بنسبة ١١.٧٪ ،٦ مصابون بمرض القلب الإقفاري بنسبة ١٠٪، ٣ مصابون بإرتجاع الصمام التاجي بنسبة ٥٪، و٢ بتمدد عضلة القلب بنسبة ٣.٣٪. حسب المدة الزمنية للمرض: وجد أن الأقل من سنتين ١٧ حالة (٢٨.٣٪)، من ٢–٣سنوات ١٧ حالة (٢٨.٣٪)، واكثر من ٣ سنوات ٢٦ حالة (٤٣.٣٪). أظهرت النتائج أن هناك ١٤ (٤٦.٧٪) من الضوابط كانت إيجابية لـــ الغلوبولين المناعي، بينما كانت ٤٩ (٨١.٧٪) حالة إيجابية لمرضي القلب. وجدت الدراسة علاقة وثيقة إحصائيا بين بين الفيروس المضخم للخلايا ومرض القلب. قيمة p (٠.٠٠١)،الخطر النسبي (٥.٠٩١) توصلت هذه الدراسة الي أن معدل الانتشار المصلي للفيروس المضخم للخلايا مرتفع في مرضى القلب والأوعية الدموية. كان هناك ارتباط كبير بين هذا الفيروس وأمراض القلب والأوعية الدموية، وبالتالي فإن الإصابة السابقة بهذا الفيروس يمكن أن تكون عامل خطر للإصابة بأمراض القلب والأوعية الدموية.

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AF	Arterial Fibrillation
AIDS	Acquired Immunodeficiency Syndrome
CAD	Coronary Artery Disease
CHD	Chronic Heart Disease
CMV	Cytomegalovirus
CVD	Cardiovascular Disease
DCM	Dilated Cardiomyopathy
EGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-Linked Immunosorbent Assay
GB	Glycoprotein B
GH	Glycoprotein H
GL	Glycoprotein L
HD	Heart Disease
HF	Heart Failure
HOPE	Heart Outcome Prevention Evaluation
HSV	Herpes Simplex Virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IHD	Ischemic Heart Disease
LDL	Low-Density Lipoprotein
LOX	Liquid Oxygen
MVR	Mitral Valve Regurgitation
PCR	Polymerase Chain Reaction
PDGF-R	Platelet Derivative Growth Factor Receptor
SPSS	Statistical Package For The Social Sciences
STEMI	St Elevation Myocardial Infarction
VHD	Valvular Heart Disease
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

CHAPTER ONE
INTRODUCTION

1.1 Background

The current study aims to examine the relative risk of Cytomegalovirus (CMV) infection in cardiovascular disease (CVD) by summarizing the currently available prospective evidence.

CVD is the leading cause of morbidity and mortality in the population worldwide. The occurrence of CVD in populations is incompletely explained by traditional cardiovascular risk factors, and the identification of additional risk factors of CVD would have profound implications for the development of new preventative strategies that could improve public health (WHO, 2018).

CMV is a DNA virus that belongs to the herpes family viruses (Britt, 2008). Cytomegalovirus infection is widely distributed in the population (Stares, *et al,* 2006). Moreover, previous studies have provided evidence that infection with CMV may play a role in the development of atherosclerosis. For example, researchers have detected CMV DNA in atherosclerotic plaques (Xenaki, *et al,* 2009).

Other studies found the level of serum CMV DNA was higher in patients with stable coronary artery disease and acute coronary syndrome than in healthy controls (Nikitskaya, *et al,* 2016) CMV IgG seropositivity was associated with future risk of stroke after adjusting for other risk factors Furthermore, there is growing evidence implying an important role of this virus in vascular pathology by introducing slow but persistent inflammation in the vessel wall (Popovic, *et al,* 2012).

Despite these studies, whether CMV infection increases the relative risk of CVD remains uncertain. Researchers found that CMV IgG seropositivity is not associated with the incidence of CVD during 10 years of follow- up, On the contrary, CMV IgG seropositivity was associated with a slight excess risk of subsequent myocardial infarction, stroke, or cardiovascular death in the Heart outcomes prevention evaluation (HOPE) study patients (Smieja, *et al,* 2003) While several studies found that CMV infection is linked to a higher relative risk of CVD (Savva, *et al*, 2013) negative results were also reported in other studies (Haider, *et al*, 2002).

1.2 Rationale

CMV is a herpes virus that causes one of the most common infections in adults, with a seroprevalence of 60-90% of the population (Zuhair, *et al,* 2020). Once acquired, the

infection persists lifelong and may undergo periodic reactivation, especially in immunosuppressed subjects. The first observations of a possible correlation between CMV and atherosclerosis were made almost 30 years ago. The presence of anti-CMV IgG antibodies within the sera and the presence of CMV antigens within vascular smooth muscle cells were associated with different stages of plaque formation in patients undergoing vascular surgery at that time (Melnick, *et al*, 1983*).*

Seroepidemiologic studies have suggested a 2-fold risk of heart disease among subjects with prior infection with CMV (Blankenberg, *et al*, 2001).

CMV direct detection by DNA or antigen identification strongly associates viral infection with atherosclerosis and cardiovascular disease as an end-point. Recent studies showed a higher viral load in STEMI patients compared to controls (Lebedeva, *et al*, 2019).

A higher number of CMV-DNA copies were detected in patients with preexistent cardiovascular risk factors and were associated with the acute coronary syndrome, suggesting that CMV reactivation may lead to the progression of atherosclerotic lesions, for example, transforming stable angina into unstable angina or myocardial infarction (Nikitskaya, *et al*, 2016).

This review highlights that CMV infection may be associated with acute atherosis, atherosclerosis, and/or cardiovascular disease in almost all population groups. It may produce vascular injuries ever since placentation and then continue to maintain a chronic

Inflammation status with subsequent vascular impairment and cardiovascular events later

in life, in both healthy and immunosuppressed patients (Cristescu, *et al*, 2022).

I, therefore, conducted a case-control study to investigate the role of the previous infection with CMV on the risk of cardiovascular disease, carefully controlling for other potentially confounding factors.

1.3 Objectives

1.3.1 General objective

to detect association between cytomegalovirus lnfection and cardiovascular diseases in patients attending sudan heart centre .

1.3.2 Specific objectives

1. To detect the CMV IgG using enzyme-linked immunosorbent assay (ELISA)
2. To correlate the possible risk factors associated with CMV seropositivity and heart disease.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO
LITERATURE REVIEW

2. 1 Background

Approximately 17.5 million people die each year from cardiovascular diseases (CVDs) worldwide. Accounting for31% of total deaths, it has been estimated that >75% of cardiovascular deaths occur in low‐ and medium-income countries (WHO, 2018). CVDs act as a large financial burden on the economy of the nation not only due to the direct cost involved in the treatment of the disease but also due to the lack of productivity associated with the disease. The combined direct and indirect cost of CVDs in the United States was estimated to be $444 billion in 2010. This staggeringly high number corresponds to $1 out of every $6 spent on health care (CDC, 2018).

Given the high global burden of CAD, prevention through identifying and mitigating risk factors is a priority. Although it is well known that hypertension, hypercholesterolemia, decreased high-density lipoprotein cholesterol, smoking, family history, and diabetes were regarded as absolute risk factors, they cannot explain all cases of atherosclerosis. Given the influx of inflammatory cells such as T‐ cells, B‐ cells, macrophages, and polymorphonuclear neutrophils in atherosclerotic plaques, inflammation has been proposed to be one of the driving forces in the pathogenesis of CAD (Tousoulis, *et al*, 2008).

The role of infective agents in triggering the inflammatory process is underscored by the relatively high prevalence of CAD in low‐ and middle-income countries, which often suffer from high rates of infection and poor sanitary and hygienic conditions. Apart from expediting the inflammatory process, infective agents can also lead to final complications of these plaques such as plaque rupture and thrombosis, introducing the concept of pathogen burden and demonstrating that the number of infectious pathogens to which an individual has been exposed is related to the presence of CAD (Zhu, *et al*, 2000).

Several studies have shown an association between previous infections with Chlamydia pneumonia, (Joshi, *et al*, 2013) herpes simplex virus (HSV), CMV, Helicobacter pylori, (Yu Xu, *et al*, 2017), and hepatitis virus or respiratory tract infection and the presence of heart disease, whereas other studies have not shown such an association (Mundkur, *et al*, 2012), (Heltai, *et al*, 2004), *(*Ridker, *et al*, 2017).

I have done this review to elucidate the pathogenesis of CMV in causing CVD. Furthermore, I have presented various studies and their association with cardiovascular diseases. By understanding the pathogenesis, we can prevent the progression of the disease.

2.2 Cytomegalovirus

CMV infects the majority of people on the planet, usually without causing any noticeable symptoms. It came to medical attention when the characteristic owl's eye inclusions were seen in stillbirths (1910) and again in (1964) among patients undergoing pioneering organ transplantation (Hill, *et al*, 1964). CMV is a prevalent opportunistic infection in the fetus, allograft patients, bone marrow transplant patients, and AIDS patients, according to more modern diagnostic procedures. In addition to causing overt end-organ damage (direct effects), it is statistically linked to indirect effects such as graft rejection and cardiovascular disease after transplantation (Beam and Razonable, 2012).

The chronic effects of this virus evading an efficient cell-mediated and humoral immune response have recently been linked to increased mortality in the general population, indicating that this virus is not as harmless as it appears (Britt, 2008).

2.2.1 Epidemiology

Antibodies of the IgG class, which indicate the previous infection, are found in 60-90% of the population. As a fetus, a neonate, a toddler, a child, or an adult, you can get infected (Zuhair, *et al,* 2019). People born into low-income families are more likely to contract CMV than those born into higher-income families (Pembrey, *et al*, 2013).

By the time of delivery, approximately 2% of seronegative women who enter pregnancy have been seroconverted. Young children, particularly toddlers, are a major source of CMV for such women, as their saliva and urine contain high levels of CMV (Cannon, *et al,* 2011).

Among the women with primary infection during pregnancy, 32% transmit the virus across the placenta to produce intrauterine infection. This is usually not recognized until the mother's symptoms are severe enough for her to seek medical attention, though retrospective examination can reveal symptoms of fever and malaise in a proportion of cases, If symptoms are investigated during pregnancy, seroconversion

or the presence of IgM antibodies in conjunction with low-avidity IgG antibodies can be used to confirm primary infection (Kenneson and cannon, 2007).

Mothers may wish to elect for amniocentesis to diagnose intrauterine infection and PCR testing of amniotic fluid for CMV DNA. When performed after 21 weeks of gestation and at least 6 weeks after the presumed time of maternal infection, this test is reliable; this demonstrates that it takes time for CMV to cross the placenta from the maternal sites of infection and for fetal kidney function to become established enough for viremia to be detectable by sampling amniotic fluid. Because the risk of severe disease caused by intrauterine infection is about 20%, many mothers choose to continue the pregnancy unless ultrasonography reveals structural damage to the fetus (Revello, *et al*, 2011).

2.2.2 Cellular and molecular biology

CMV's broad cellular tropism is likely to contribute to the wide range of pathologies associated with infection. Despite the presence of multiple membrane glycoproteins in the virion envelope, only two complexes have been identified as being required for entry (Vanarsdall and Johnson, 2012).

The absence of glycoprotein B (gB) prevents CMV from entering cells unless a chemical fusogen (e.g., polyethylene glycol) is present (Isaacson and Compton, 2007). These findings, along with previous research on gB in other herpes viruses, indicate that gB is required for virus fusion. Furthermore, gB participates in the initial cell attachment via interactions with heparan sulphate glycosaminoglycans. CMV gB has also been shown to interact with a number of cellular receptors, including EGF-R, PDGF-R, tetherin, and integrins (21, 61, and v3), which can affect infection efficiency in a strain- and cell type-dependent manner, though the evidence that these are bona fide mediators of entry remains debatable. The second complex is the gH–gL dimer, which is also required for entry into all cell types and is thought to be involved in the activation of gB fusion at the plasma membrane. Surprisingly, this dimer also forms a pentameric complex with three other viral proteins (UL128, UL130, and UL131) that are required for efficient entry into endothelial and epithelial cells but redundant for fibroblast infection. Finally, targeting the activity of gB and gH–gL complexes (via neutralizing antibodies and vaccines) is regarded as a critical strategy for controlling CMV in a variety of settings where exogenous infection poses a clinical risk (Hahn, *et al*, 2009).

2.2.3 Pathogenesis

Studies of the replication rate of CMV showed that its dynamics are rapid, with a doubling time of viremia of approximately one day. This is very similar to those of primary HIV infection (Emery, *et al.* 2002).

The reputation of CMV as a slowly growing virus is thus undeserved and is derived from the slow evolution of cytopathic effects in fibroblast cell cultures. We now know that strains of CMV that replicate in these cells have extensive genetic changes from the wild-type and are less pathogenic to human volunteers than wild-type viruses. Epithelial cells and endothelial cells are more representative of CMV infection in humans (Plotkin, *et al,* 1989).

The presumed pathogenesis is CMV replication stimulating the release of cytokines, which are then toxic to the lungs. A current controlled clinical trial is randomizing seropositive patients admitted to intensive care to receive ganciclovir or placebo, to determine whether this putative effect can be controlled. In elderly patients, the accumulation of decades of chronic immune surveillance for CMV hiding in sanctuary sites might produce excess mortality in two main ways. First, the reduced number of naive T cells might make seropositive people less able to respond to vaccines for influenza or pneumococcal infection, and there is some, inconsistent, evidence for this from small studies (Wald, *et al,* 2013).

Second, the increased abundance of activated T cells might mediate inflammatory attacks on bystander cells, such as those forming the endothelium, to increase the risk of cardiovascular disease. Given the major effects of CMV on cell-mediated immunity, natural killer cells, and cytokines, one might imagine that CMV could affect overall mortality (Gkrania-Klotsas, *et al,* 2013).

Most immunosuppressive drugs cause CMV end-organ disease by increasing the viral load, while steroids cause disease by lowering the viral load threshold required (Emery and Griffiths, 2000).

2.2.4 Transmission

People with CMV may pass the virus in body fluids, such as saliva, urine, blood, tears, semen, and breast milk. CMV is spread from an infected person in the following ways: from direct contact with saliva or urine, especially from infants and young children, through sexual contact, from breast milk to nursing infants, or through transplanted organs and blood transfusions (Dollard, *et al,* 2007).

CMV can only be passed on when it's "active". The virus is active when the infection is for the first time – young children often get CMV for the first time at nursery, or the virus has "re-activated" – because of a weakened immune system, or re-infection – with a different type (strain) of CMV. Pregnant women can pass an "active" CMV infection onto their unborn fetus this is known as congenital CMV (Gindes, *et al*, 2008).

During solid organ transplantation, seropositive donors frequently (approximately 78%) transmit CMV to seronegative recipients (Atabani, *et al*, 2012).

2.2.5 Signs and symptoms

CMV infection is mostly or mildly symptomatic among the general population (85-90%) Some people get flu-like symptoms the first time they get CMV, including a high temperature, aching, muscle tiredness, skin rash, feeling sick, sore throat, and swollen glands. People with weakened immunity may suffer from a problem affecting the eyes, lungs, liver, esophagus, stomach, intense and brain. Infants may show jaundice, purple skin splotches, rashes, microcephaly, enlarged spleen, pneumonia, and seizure (Dreher, *et al*, 2014).

2.2.6 Diseases

around 10%–15% of infants with the congenital infection may be at risk of sequelae such as mental retardation, jaundice, hepatosplenomegaly, microcephaly, hearing impairment, and thrombocytopenia (Fowler and Boppana, 2006).

Among the above sequelae, the most devastating one is the central nervous system (CNS) sequelae related to neurodevelopment in that CNS injury is irreversible and persists for life, including mental retardation, seizures, hearing loss, ocular abnormalities, and cognitive impairment. That means the asymptomatic newborns with CMV infection still have an increased risk for long-term sequelae, especially, mental retardation and sensor neural hearing loss (SNHL) making CMV the leading nonhereditary cause of SNHL (Nance, *et al*, 2006).

2.2.7 Laboratory diagnosis

For diagnosing CMV infection, a variety of detection methods are available including direct virus identification by cell culturing, antigen detection of the virus, detection of CMV DNA, detection of IgM and IgG antibodies, or detection of T cell responses against CMV, which can be applied depending on the problem under study. Primary diagnosis of CMV infection can be established by antigen detection (pp65), for

instance in leucocytes in the blood, saliva, and urine, as well as by means of isolating the virus or nucleic acid amplification techniques (NAT) before seroconversion (Staras, *et al,* 2008).

Seroconversion is confirmed by the detection of CMV-specific IgM and/or IgG antibodies in the serum. For this purpose, both ELISA and immunofluorescence tests are available. Diagnostics of the infection are performed serologically based on increases in IgM and IgG itre, which are measured in two serum samples drawn at an interval of about 2 weeks. The domain of the serological tests lies in the definition of the serostatus (Preiksaitis, *et al,* 2005).

Reactivation can be detected serologically by means of identifying a significant increase in the titer of CMV-specific antibodies. In addition, through avidity determination of the IgG antibodies, a distinction can be made between primary infection (low avidity and binding against multivalent antigen) and secondary infection (high avidity). The immunoblot is considered the gold standard for confirmation of IgM antibodies in the serum (Lazzarotto, *et al,* 2008).

To monitor reactivation in transplantation patients, quantitative detection of pp65 antigen in neutrophil granulocytes, and, recently to an increasing extent, quantitative CMV DNA detection from whole blood or plasma are performed. In the case of CMV encephalitis, CMV DNA can be detected in the liquor. For long-term treatment with antiviral substances (e.g. in AIDS patients with retinitis), in vitro sensitivity determination of the viruses against medicinal products used or genotype resistance determination by means of sequencing can be sensible diagnostic methods in justified individual cases. In the future, microarrays may complete CMV diagnostics (Petrik, 2006).

2.2.8 Prevention

In light of what is known about CMV transmission routes, steps to prevent infection in at-risk groups have been devised. The risk of primary infection in solid organ transplant recipients, for example, could be reduced by matching seronegative donors and recipients, though this approach is hampered by donor organ scarcity (Griffiths, *et al,* 2011).

Antiviral prophylaxis and pre-emptive therapy have proven beneficial in transplant patients. By using CMV sero-negative, filtered, or leukocyte-deprived blood products, transmission to immunocompromised individuals, pregnant women, and premature

newborns can be avoided. This method places a greater burden on blood transfusion centers, but it should be used whenever possible because it reduces CMV transmission significantly. Another method of protecting vulnerable groups would be to administer a CMV vaccine to ensure pre-exposure immunization. Because the virus is a leading cause of CNS damage in children, developing a vaccine to prevent congenital CMV infection is a top priority (Pass, 2001).

The recombinant, soluble gB vaccine with MF59 adjuvant was studied in two populations. Seronegative women who had recently given birth were randomized to receive the B vaccine/MF59 or a placebo. On follow-up, approximately 50% protection was seen against CMV seroconversion (pass, *et al,* 2009).

2.2.9 Treatment

For the past two decades, the mainstay of preventing and treating CMV infection has been through ganciclovir or valganciclovir, together with the off-label use of foscarnet in some cases. Cidofovir has activity against CMV, but its renal toxicity precludes its use in routine clinical practice. Ganciclovir (or valganciclovir) is used for pre-emptive therapy or prophylaxis in transplant patients, as described above. In AIDS patients, they are used to treat CMV end-organ disease in the few patients who develop retinitis. Ganciclovir was studied in randomized controlled trials by the Collaborative Antiviral Study Group in the USA, starting with 6 weeks of therapy to address the threshold concept, and because longer intravenous treatment would be difficult to give in neonates. Cases born with CNS symptoms were randomized to receive ganciclovir at 4 mg/kg twice daily or 6 mg/kg twice daily. Both doses produced clinically significant neutropenia, which was managed through dose reduction. The higher dose appeared to be slightly better at controlling viruria (measured by cell culture) and was selected for a phase III trial. Neonates born with symptoms involving the CNS were randomized to receive ganciclovir for 6 weeks or no treatment. The results showed a reduction in sensor neural hearing loss measured at 6 months and in the number of missed developmental milestones, so this treatment was rapidly adopted as the standard of care (Oliver, *et al,* 2009).

The investigators then showed that valganciclovir was bioavailable in neonates, opening the way for longer treatment durations (Acosta, *et al,* 2007).

Another phase III trial gave valganciclovir 16 mg/kg twice daily for 6 weeks to all neonates born with symptoms (not necessarily involving the CNS) and then

randomized them to receive valganciclovir or a matching placebo to complete a 6-month course (Kimberlin, 2014).

The results showed improved control of sensor neural hearing loss and developmental milestones among those who received the longer course. Interestingly, the incremental benefit of the much longer course was less than the four-fold increased duration of drug exposure, consistent with the substantial benefit being obtained by inhibition of the threshold effect. Future correlation of clinical outcomes with CMV viral load in urine, saliva, or blood has the potential to refine such studies. Three new antiviral drugs have entered clinical trials recently. All were studied in phase II by determining whether, compared to placebo, prophylaxis could decrease the need for pre-emptive therapy after a stem cell transplant. Maribavir inhibits the UL97 enzyme, which breaks down the nuclear lamina to allow the egress of virions into the cytoplasm. After a successful phase II study, the endpoint of CMV end-organ disease was selected for a phase III trial (Winston, *et al,* 2008).

This showed no advantage of maribavir over placebo because all patients with viremia were given pre-emptive therapy with ganciclovir, which masked any difference between the study arms. In addition, the dose chosen for phase III was probably too low (Marty and Boeckh, 2011).

Maribavir is now being examined, at a higher dose, for potency as pre-emptive therapy. Brincidofovir is a lipid prodrug of cidofovir that is rapidly taken up into cells and is not a substrate for the oxyanion transporter in the kidney. As a result, this prodrug does not have the renal toxicity of the parent compound, although gastrointestinal toxicity is dose-limiting (Marty, *et al,* 2013).

Letermovir inhibits the terminase enzyme complex that normally cleaves concatameric DNA into unit lengths as it is packaged into the virion (Chemaly, *et al,* 2014).

None of these drugs showed bone marrow toxicity, so the paradigm of delaying prophylaxis until the time of engraftment may, in the future, become a historical relic of the era when only ganciclovir was available. At the preclinical stage, the discovery that latent HCMV is associated with inhibition of the multidrug-resistant protein suggests the possibility of using cytotoxic compounds to destroy cells containing latent protein (Weekes, *et al,* 2013).

2.3.1 Role of CMV in cardiovascular disease

CMV can cause atherosclerosis both through direct and indirect mechanisms. Direct mechanisms involve the pathogenesis of atherosclerosis because of the gene products of CMV. CMV infection of endothelial cells generates gene products IE72 and IE84 that activate the COX‐2 promoter. This increases the activity of the nuclear factor‐κB (NF‐κB) transcription factor through increased reactive oxygen species production. Increased NF‐κB further mediates the expression of adhesion molecules such as ICAM‐1, VCAM‐1, VAP‐1, and E‐selectin on the endothelial cells. The increased expression of adhesion molecules favors the adhesion of monocytes/macrophages to the endothelial cells and contributes to atherosclerosis. Furthermore, increased levels of NF‐κB also make the atherosclerotic plaque unstable by releasing matrix metalloproteinase‐9. Smooth muscle migration and proliferation is an important steps in the formation and progression of atherosclerotic plaque. Infection with CMV expresses the US28 chemokine receptor (Vomaske, *et al*, 2009).

This chemokine receptor promotes the migration of smooth muscle cells to tunica intima. In addition, the CMVIE84 gene product generated in endothelial cells inhibits the transcriptional activity of p53. (Tanaka, *et al*, 2017).

The down-regulation of p53 increases the proliferation of smooth muscle cells by inhibiting apoptosis and thus contributes to the propagation of atherosclerosis.

Indirect mechanisms are due to the production of cytokines at far off place that mediates atherosclerosis through effects other than direct involvement of CMV gene products. Studies have shown that infection with CMV raises the level of pro‐inflammatory cytokines, namely interferon‐gamma and tumor necrosis factor‐alpha (TNF‐α) Increased titer of pro‐inflammatory cytokines increases the risk of rupture of plaque and makes the patient prone to CAD and its complications. TNF‐α further transactivates NF‐κB and thus, predisposes to atherosclerosis by the mechanisms described above.

In diabetic patients with poor glycemic control, there is an increased risk of CMV infection in the arterial wall. In addition, can also act as a pro‐thrombotic agent and enhance the production of thrombin and thus activating the coagulation cascade. (Sherman, *et al*, 2014).

Increased thrombin generation worsens the risk of atherosclerosis and CAD. Herpes simplex virus 1 and 2HSV‐1 and 2 members of the herpesvirus family are double‐stranded DNA viruses that can cause a wide range of presentations from benign cutaneous oral or genital lesions to severe HSV encephalitis. Multiple studies link an association between HSV‐1/2 infection and atherosclerosis. HSV promotes atherosclerosis by acting on endothelial cells, vascular smooth muscle cells (VSMCs), and macrophages. Endothelial cells can act as a site of latency for the virus, which may undergo periodic reactivation and cause enhanced atherosclerosis. HSV infection on the vascular endothelium causes an increase in pro‐thrombotic activity and thereby atherosclerosis by various mechanisms first, HSV infection decreases the synthesis of heparan sulfate proteoglycan that impairs the action of antithrombin 3 necessary for inactivating the activated coagulation factors, thus increasing the pro‐thrombotic activity. Second, the infection by HSV causes impaired thrombomodulin surface expression on the endothelium that reduces the levels of protein C, further contributing to increased thrombotic activity and thus atherosclerosis. Third, HSV infection can increase thrombin generation and decrease the levels of prostacyclin, thus, increasing platelet adhesion. In addition, HSV infection of endothelial cells induces the cell surface expression of P‐selectin (GMP 140) and von Willebr and factor that promote the adhesion of monocytes and platelets to the endothelium, favoring thrombosis and atherosclerotic plaque formation. HSV‐1 infection of VSMCs causes the accumulation of saturated cholesterol esters (CEs) and triacylglycerols (TAGs) that predispose to atherosclerosis. There are various mechanisms responsible for the accumulation of CEs and TAGs including increased low‐density lipoprotein (LDL) binding and uptake, increased LDL receptor gene transcription, increased 3‐hydroxy‐3‐methylglutaryl‐coenzyme A reductase activity, and decreased CE hydrolase activity (Etingin, *et al,*1991).

In addition, infection with HSV induces the expression of HSV glycoprotein that stimulates platelet‐derived growth factor production and promotes smooth muscle cell proliferation, thereby enhancing atherosclerotic plaque formation. Furthermore, HSV‐1 infection also upregulates the expression of LOX‐1 receptor on the macrophages, and thus, there is enhanced uptake of oxidized LDL promoting foam cell formation and thereby promoting atherosclerosis (Chirathaworn, *et al,* 2004).

2.3.2 Cardiovascular disease in Sudan

The WHO estimates that a total of 57 million deaths occurred worldwide in 2008, 36 million of which were due to non-communicable diseases. The burden of cardiovascular disease states is stabilizing in high-income countries, while in low-to-middle-income countries it continues to rise. Over the past 55 years in West Africa, there has been a 20% decrease in communicable diseases, which has been offset by a proportionate increase in non-communicable diseases, particularly cardiovascular disease (van der Sande, *et al,* 2001).

Sub-Saharan African countries are currently experiencing one of the most rapid epidemiological transitions characterized by increasing urbanization and changing lifestyle factors. This has resulted in an increase in the incidence of non-communicable diseases, especially cardiovascular disease. (Steyn, *et al,* 2005) noted that globally, including sub-Saharan African countries, 90% of cardiovascular risk factors include smoking, alcohol consumption, obesity, diet, low physical activity, psychosocial factors, diabetes mellitus, hypertension, and high lipid levels. Cardiovascular disease is strongly influenced by socioeconomic status in all societies, whether one considers accepted risk factors, heart disease, hypertension, or stroke. As a population, blacks have one of the highest rates of coronary artery disease in the world. Hypertension is widely recognized as a major cause of cardiovascular morbidity and mortality in indigenous people of Africa (Cruickshank, *et al,* 2004).

Furthermore, several studies have shown that male urban dwellers in Africa have a higher incidence of hypertension compared to males living in rural areas. Hyperhomocysteinaemia is associated with an increased risk of cardiovascular disease that can lead to stroke or heart attack, both of which are causes of mortality in African populations, especially males (Mtabaji, *et al,* 1990).

The prevalence of cardiovascular disease phenotype and risk factors included ischemic heart disease, cardiomyopathy, endomyocardial fibrosis, rheumatic heart disease, congenital heart disease, angina, stroke, diabetes mellitus, hypertension, and kidney disease were recorded. The family history including heart attack, angina, stroke, diabetes mellitus, and hypertension were determined (Glew, *et al,* 2002).

2.3.3 Clinical characteristics and modifiable risk factors

The risk for cardiovascular disease in Sudanese patients could be multiple, ranging from social, economic, lifestyle (smoking, sedentary lifestyle, improper diet) and

biological (abnormal lipids, hypertension, diabetes, obesity). In the present study, 12.19% of patients are past users of tobacco and 1.63% are alcohol drunker. The prevalence of cardiovascular disease phenotype and risk factors included ischemic heart disease (57.72%), cardiomyopathy (32.52%), endomyocardial fibrosis (4.88%), rheumatic heart disease (17.89%), congenital heart disease (9.76%), angina (16.26%), stroke (3.25%), diabetes mellitus (27.64%), hypertension (44.72%) and kidney disease (8.13%). The family history of heart attack, angina, stroke, diabetes, and hypertension for patients was 12.19%, 13.01%, 3.25%, 37.39%, and 43.90%, respectively (Musa, *et al,* 2018).

2.4 Previous Studies

Studies in developing countries such as India (Kothari, *et al,* 2002), Iran (Shaiegan, *et al,* 2015), Saudi Arabia (Al‐ Ghamdi, *et al,* 2011), and Turkey (Ocak, *et al,* 2007) reported a positive association between CMV and development of CAD. However, many studies in developed countries such as Hungary (Heltai, *et al,* 2004), Germany (Ridker, *et al,* 2017), and the USA (Zhu, *et al,* 2000) reported no association between CMV infection and CAD. This is consistent with higher seroprevalence of CMV in developing countries and lower seroprevalence in developed countries.

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study type and design: The study was a case-control study.

3.2 Study area and duration: This study was conducted in Khartoum Heart Center, Khartoum, Sudan. The study was conducted from June to September 2022.

3.3 Study population: Patients with heart disease of any age and apparently healthy control group were included.

3.4 inclusion criteria : all patients with cardiovascular diseases.

3.5 exclusion criteria :Patients born with congenital heart disease.

3.6 Sample size: Ninety (90) study populations enrolled in this study, 60 chronic heart disease Patients as a case group and 30 healthy subjects as a control group.

3.7 Sample technique: Non-probability convenience.

3.8 Ethical considerations: The ethical clearance was approved by the Ethical and Scientific Committee of Medical Laboratory Science College, Sudan University of Science and Technology; permission to carry out the study was taken from the Sudan heart center. Verbal consent from each study participant was taken.

3.9 Data collection: Data collected using a structured questionnaire that includes demographic information such as age, gender, and duration of heart disease.

3.10 Laboratory methods

3.10.1 Collection of samples: Serum specimen collection: The blood specimens were collected using venipuncture for collection. (5ml) ml of blood was collected using a sterile syringe after cleaning the skin area with alcohol pads and the blood was dispensed in a sterile plain container. Blood specimens after clotting were centrifuged at 3000 rounds/minute for 5 minutes to obtain serum, and then the obtained sera were collected in clean sterile containers properly labeled and kept at -20 ºC till used.

3.10.2 Detection of CMV IgG using ELISA: The CMV IgG ELISA Test Kit is a solid phase enzyme immunoassay based on an indirect principle for the qualitative detection of IgG antibodies to CMV in human serum or plasma. The microwell plate is coated with CMV antigens. During testing, the specimen is diluent, and the specimens are added to the antigen-coated microwell plate and then incubated. If the specimens contain IgG antibodies to CMV, they will bind to the antigens coated on the microwell plate to form immobilized antigen-CMV IgG antibody complexes. If

the specimens do not contain IgG antibodies to CMV, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated anti-human IgG antibodies are added to the microwell plate and then incubated. The enzyme-conjugated anti-human IgG antibodies will bind to the immobilized antigen-CMV IgG antibody complexes present. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of CMV IgG antibodies present in the specimens. The sulfuric acid solution was added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of CMV IgG antibodies present in the specimens, was measured with a microplate reader at 450/630-700 nm or 450 nm.

3.11 Data analysis: Collected data were analyzed using the statistical package of social science (SPSS) v25. a *P-value* of <0.05 is considered significant.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of 90 patients with heart diseases participated in this study divided into two groups; 60 (66.7%) were in the cases group, and 30 (33.3%) were in a control group. The age range of the study group was between 13 to76 years with the majority above 55 years, and the mean age was 49.9 years (Figure 4.1).

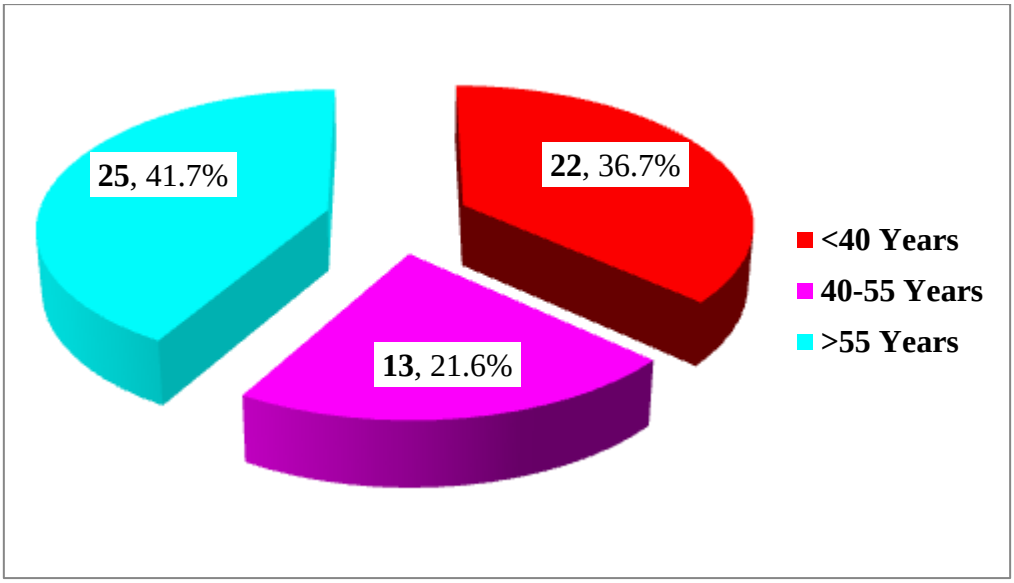

Figure 4.1: Age distribution

However, 35 (58.3%) of the population were males, and 25 (41.7%) were females as shown in (Figure 4. 2).

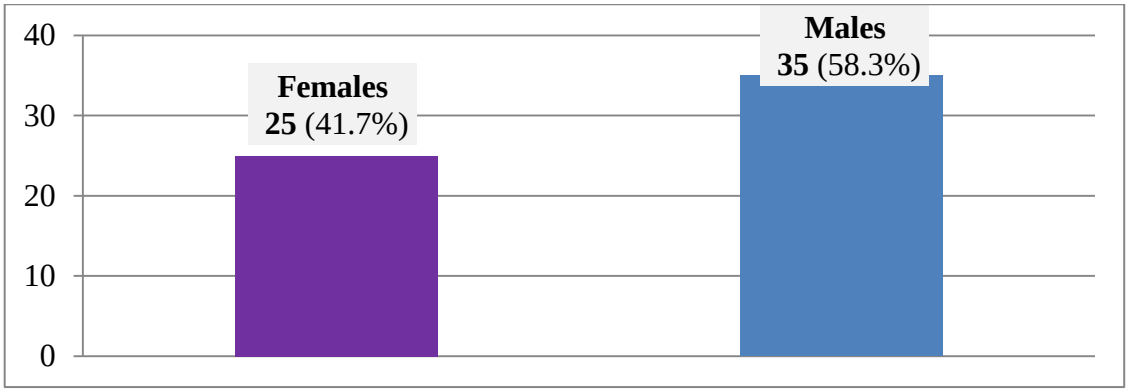

Figure 4.2: Gender distribution

Most of the patients presented with CAD 15 (25%), AF 10 (16.7%), HF 9 (15%), increase in heart palpitations 8(13.3%), VHD 7(11.7%), IHD 6(10%), MVR 3(5%), DCM 2(3.3%), (Figure 4.3).

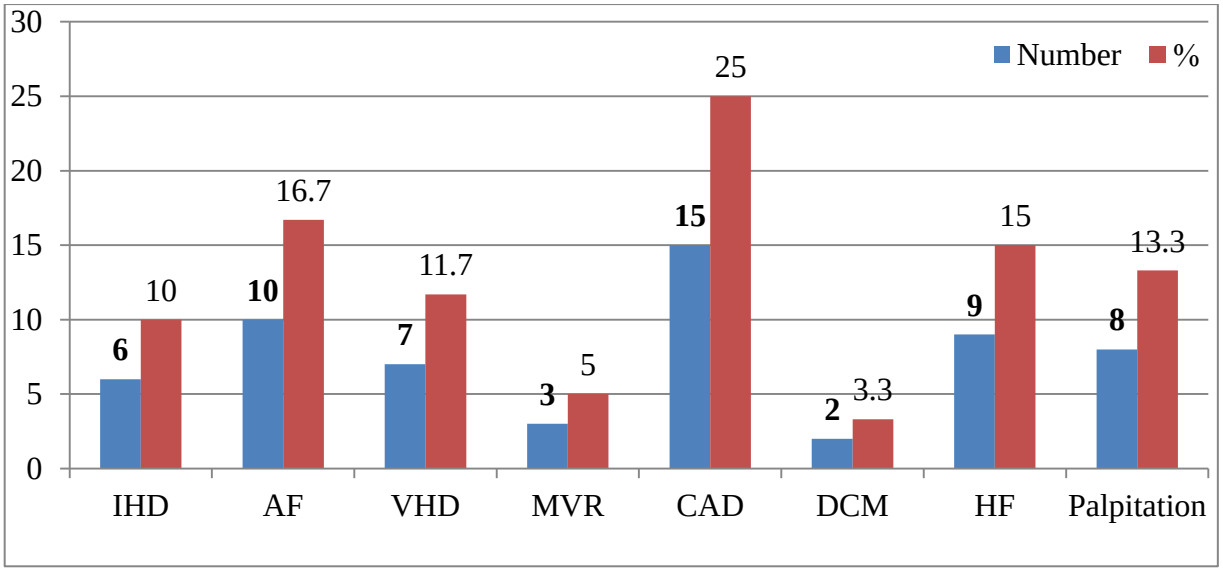

Figure 4.3: Distribution of different types of CVD

According to the duration of diseases <2 years had 17(28.3%), 2-3 years had 17(28.3%), and >3 years had 26 (43.4%) (Figure 4.4.)

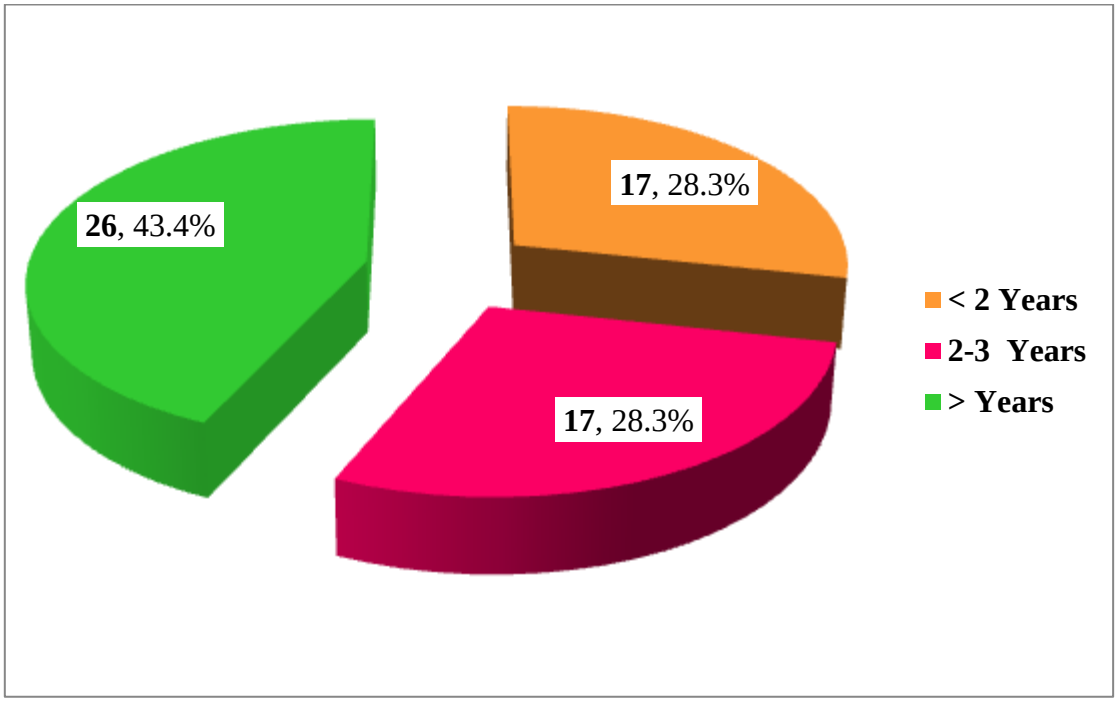

Figure 4.4: Distribution of the duration of CVD

The ELISA results of CMV revealed that there were 14 (46.7%) controls were positive for IgG, while49 (81.7%) cases were positive for IgG from a total of 60 patients in the case group, and 30 control group (Figure 4.5).

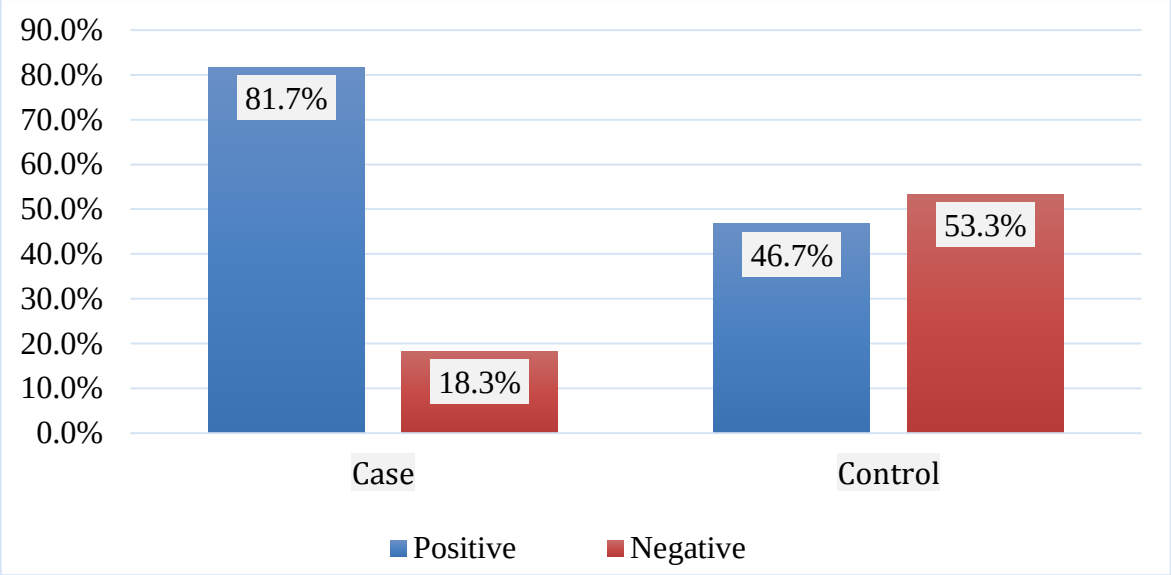

Figure 4.5: CMV IgG results for case and control

There was a significant difference in CMV IgG between cases and controls with a *P. value* (0.001) (Table 4.1).

Table No 4.1: Association of CMV seropositivity between case and control groups

	Results		Total	*P. value*	RR
	Positive	**Negative**			
Case	49 (54.4%)	11 (12.2%)	60 (66.7%)	0.001	5.091
Control	14 (15.6%)	16 (17.8%)	30 (33.3%)		
Total	**63 (70.0%)**	**27 (30.0%)**	**90 (100.0%)**		

Moreover, there was no significant difference in age groups with a *P. value* (0.366). also, there was no association in gender, duration, and disease groups with *P. values* of 0.102, 0.998, and 0.703 respectively (Table 4.2).

Table No 4.2: Association between CMV seropositivity and study variables

		Results		Total	P. value
		Positive	Negative		
Age	< 40	18 (30.0%)	04 (06.7%)	22 (36.7%)	0.366
	40 - 55	09 (15.0%)	04 (06.6%)	13 (21.6%)	
	> 55	22 (36.7%)	03 (05.0%)	25 (41.7%)	
Gender	Male	31 (51.7%)	04 (06.7%)	35 (58.3%)	0.102
	Female	18 (30.0%)	07 (11.7%)	25 (41.7%)	
Duration	< 2	14 (23.3%)	03 (05.0%)	17 (28.3%)	0.988
	2 – 3	14 (23.3%)	03 (05.0%)	17 (28.3%)	
	> 3	21 (35.0%)	05 (08.4%)	26 (43.4%)	
Disease	IHD	06 (10.0%)	00 (00.0%)	06 (10.0%)	0.703
	AF	08 (13.3%)	02 (03.3%)	10 (16.7%)	
	VHD	06 (10.0%)	01 (01.7%)	07 (11.7%)	
	MVR	03 (05.0%)	00 (00.0%)	03 (05.0%)	
	CAD	12 (20.0%)	03 (05.0%)	15 (25.0%)	
	DCM	02 (03.3%)	00 (00.0%)	02 (03.3%)	
	HF	07 (11.7%)	02 (03.3%)	09 (15.0%)	
	Palpitation	05 (08.3%)	03 (05.0%)	08 (13.3%)	

CHAPTER FIVE

DISCUSSION, CONCLUSION, RECOMMENDATIONS

CHAPTER FIVE

DISCUSSION, CONCLUSION, RECOMMENDATIONS

5.1 DISCUSSION

In this case-control study, We found evidence that past infection with CMV was significantly associated with an increased risk of CVD, this study is in agreement with (Wang, *et al,* 2017), in their meta-analysis of 10 prospective cohort studies, they reported that CMV seropositivity was associated with a 22% (relative risk [RR] = 1.22; 95% CI, 1.07–1.38) increased risk of CVD compared to seronegative individuals, estimated that 13.4% (95% CI, 12.0%–14.5%) of CVD events could be attributed to CMV. This is larger than other well-established modifiable risk factors such as smoking, poor diet, low education, obesity, and diabetes, which were each reported to have a CVD population attributable fraction of 5% to 6% in a cohort of 155 722 people from 21 high-, middle-, and low-income countries.

In contrast, many studies from developed countries (Hamilton, *et al*, 2022), (Roberts, *et al*, 2010), (Sorlie, *et al,* 2000) show that there is no association between CMV and cardiovascular diseases. This is consistent with higher seroprevalence of CMV in developing countries and lower seroprevalence in developed countries. Countries such as India, Iran, Saudi Arabia, and Turkey have reported >80% seropositivity of CMV, while European and North American countries have seropositivity ranging from 30% to 55%.

The age group >55 years had higher CMV seropositivity (36.7%) than other age groups which agrees with (Schiele, *et al,* 2001) whom reported that CMV is more seropositivity increased with age.

In the study the seroprevalence of CMV is higher in males than in females (51.7%), this agrees with (Schiele*et al,* 2001) who reported 87% CMV infection with heart disease in males, and (Adam *et al,* 1987*)* reported higher seroprevalence in males with atherosclerosis, while (Kim, *et al,* 2015) and (Hamilton, *et al,* 2022) reported that females with CVD had higher seropositivity to CMV than males with Seroprevalenceof about 58%.

This study also showed that patients with CVD for more than 3 years had higher seroprevalence of CMVcompared to other groups.

In this study, the seropositivity of CMV within the types of disease were 62% for palpitation,77% for HF, 80% for AF and CAD,85% for VHD, and 100% for IHD and MVR.

Several studies in developing countries such as India (Kothari, *et al*, 2002), Iran (Alavi, *et al*, 2011), Saudi Arabia (Al‑ Ghamdi, *et al*, 2011), and Turkey (Eryol, *et al*, 2005) reported a positive association between CMV and development of CAD. However, studies in developed countries such as Hungary (Heltai, *et al*, 2004), Germany (Ridker, *et al,* 2017), and the USA (Zhu, *et al,* 2000) reported no association between the two variables.

In this study, all IHD patients were seropositive to CMV IgG (10%).

Gkrania-Klotsas, *et al*, 2012 reported that CMV seropositive participants with the highest antibody titer were at a 21% (95% CI, 4%–41%) increased risk of IHD compared to the seronegative participants. Two small case-control studies by (Strachan, *et al,* 1999)and (Sorli, *et al*, 2000)described no significant U-shaped associations across CMV quartiles with IHD. However, whilst CMV might be a modifiable risk factor for CVD, (Hamilton, *et al,* 2022) in their study did not find evidence of a significant association between CMV, CVD, IHD, and stroke.

Much of this existing evidence is limited by small sample size, and therefore to elicit the true shape of any association, more power is needed. Furthermore, to enable meaningful comparison and synthesis of existing evidence in relation to dose-response associations between CMV antibodies and CVD, the use of standardized CMV assays and methods for generating thresholds to categorize CMV antibodies is needed. Finally, this study, small in size compared to existing studies, may not have the power to reliably detect modest effects of CMV on CVD. Given the magnitude of the public health burden associated with CVD worldwide, it is important that such modest effects of CMV on CVD are reliably described.

5.2 CONCLUSION

In the study population patients with CVD showed high Seroprevalence of CMV IgG antibody. So, there is an association between CMV infection and CVD in the study population. CMV infection can promote vascular inflammation.

5.3 RECOMMENDATIONS

1. Another investigation like PCR is needed for more confirmation with an increased sample population.
2. Further studies with a larger sample size should be done to estimate the infection with CMV among CVD patients.

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Sudan university of science and technology

Collage of graduate studies

Association between Cytomegalovirus Infection and Cardiovascular Diseases in patient attending Sudan Heart Center

Questionnaire

ID Number ( )

Age ( ) years

Gender : male ( ) Female ( )

Disease type :

IHD ( ) AF( ) VHD ( )

MVR ( ) CAD ( )

HF ( ) Palpitaion ( ) DCM ( )

Duration of disease :

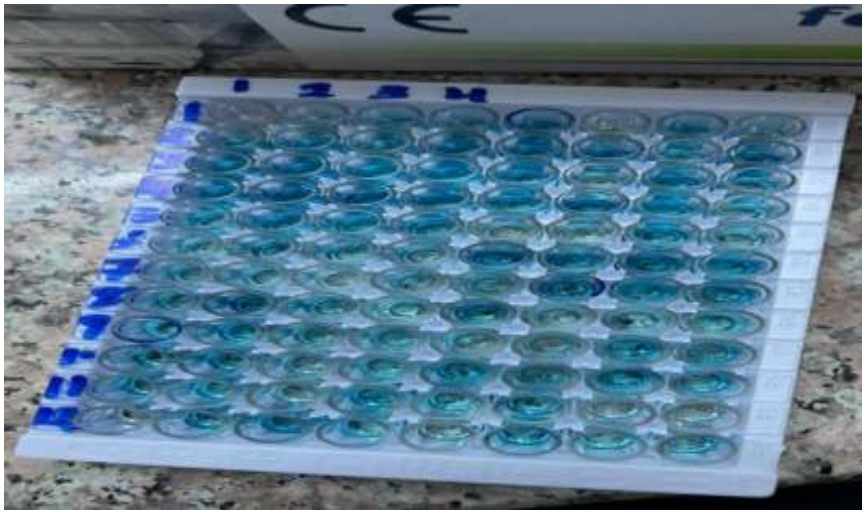
Less than 2 years ( ) 2-3 years ( )

More than 3 years ( )

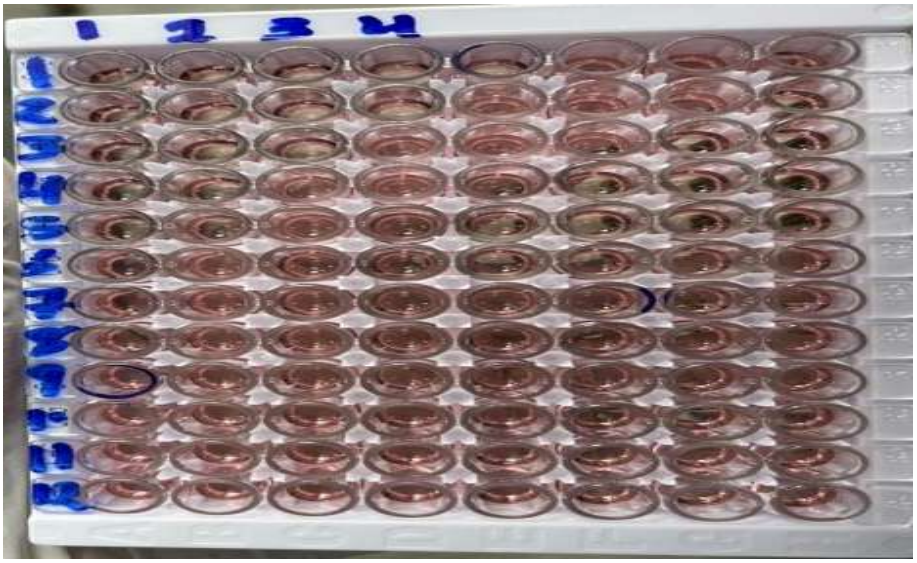
APPENDICES

CMV IgG ELISA kit

Steps of sample preparation for ELISA analysis

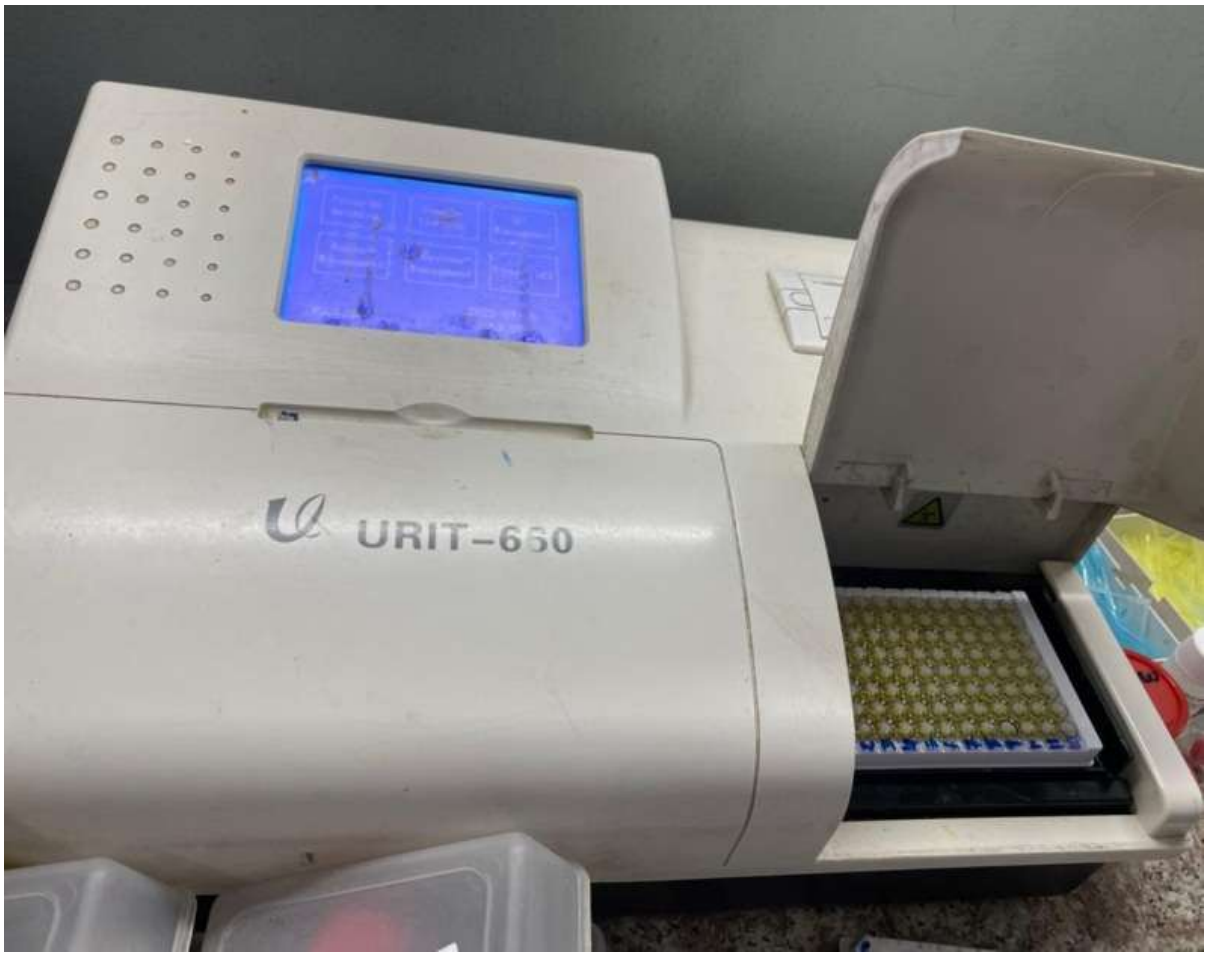


1. Adding samples



2. Conjugate

3. Stop solution



ELISA Machine