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Relationship between Cytomegalovirus (CMV) and Breast Cancer among Sudanese's Ladies in Khartoum State

Adissertation submitted in partial fulfillment for requirements of MSc Degree in Medical Laboratory Sciences (Microbiology)

By

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الآيــه :

قال تعالى:

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

(اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ ۖ الْمِصْبَاحُ فِي زُجَاجَةٍ ۖ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ ۚ نُورٌ عَلَىٰ نُورٍ ۗ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ)

صدق الله العظيم

سورة النور{ 35}

Dedication

To whom did you suffer, I learned and changed..

To those who said it was impossible and I believed in hope.

To myself

To who knocked the doors of the seventh heaven in the dead of night by the most earnest invitations to me, and the thinner of tears shed my love for me,

To the light of the eyes of my dear mother Ahlam.

To who planted in my soul the hope and spirit of life and struggle, and that it is not impossible to exist, a permanent guide to my dear father mawia.

To whom I have forged with them the most beautiful memories and the best wishes.

To those who listened to me, advised me and shared my tears before I laughed.

To my friends and fellow companions.

To my wife , my dear sons in the future.

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List of abbreviation

abbreviation	Explanation
ELFA	Enzyme Linked Fluorescent Assay
HCMV	Human Cytomegalovirus
CMV	Cytomegalovirus
IgG	Immunoglobulin G
SPR	Solid Phase Receptacle
SSPS	Statistical package for social sciences
EBV	Epstein Barr Virus
CNS	Central Nervous System
OTC	Over The Counter
HHV	Human Herpes Virus
HIV	Human Immunodeficiency Virus

Abstract

Background The breast cancer most common cancer among women. Numerous genetic and environmental factors may play a role in the development of breast cancer. Suggests that viruses may play a role in development of breast cancer. *Herpesviridae* family has been implicated as a cause of breast cancer. Human cytomegalovirus [HCMV] and Epstein–Barr virus (CMV) could potentially involve in breast cancer.

Objective: relationship between CMV IgG concentration and breast cancer among Sudanese ladies

Materials and Methods: In this analytical case control study, evaluated 50 patients with breast cancer and 50cases as control group who were referred in Medical Centre. Patients with pathologic confirmation of breast cancer were selected. Detection of CMV IgG in blood serum by using the technique ELFA (Enzyme Linked Fluorescent Assay).

Results: In this study, 50 patients with breast cancer participated. Patients with mean age of (42.5 ± 10.34) years. CMV IgG was positive in 49 (98%) patients P. Value of the relation between the CMV IgG and patient's age is (0.03) and the P. Value of the relation between social status of patients and IgG concentration = (0.09)

Conclusion: there has association between the CMV IgG and patient's age , social status of patients with the IgG concentration.

Key word: CMV , Breast cancer , IgG , Center of Khartoum.

المستخلص

مقدمة؛ كان السرطان الأكثر شيوعا بين النساء عالميا سرطان الثدي. قد تلعب العديد من العوامل الجينية والبيئية دوراً في تطور سرطان الثدي. تشير إلى أن الفيروسات قد تلعب دورا في تطوير سرطان الثدي. وقد , ثبت بأن عائلة *Herpesviridae* كسبب لسرطان الثدي. الفيروس المضخم للخلايا البشري [HCMV] و (CMV) يمكن أن ينطوي على احتمال الإصابة بسرطان الثدي.

الهدف؛ العلاقة بين تركيز CMV IgG وسرطان الثدي بين السيدات السودانيات.

المواد والطرق؛ في هذه الدراسة التحليلية دراسة الحالة ، قيمت 50 مريضا بسرطان الثدي و 50 حالة كمجموعة الضابطة الذين أحيلوا في المركز الطبي - الخرطوم - السودان من أكتوبر 2017 إلى يونيو 2018. تم اختيار المرضى الذين يعانون من سرطان الثدي. الكشف عن CMV IgG في مصل الدم باستخدام تقنية ELFA (مقايسة الفلورسنت المرتبط بالإنزيم).

النتائج؛ في هذه الدراسة ، شارك 50 مريضا. متوسط عمر المرضى(42.5 ± 10.34) سنة. كان CMV IgG إيجابيا في 49 مريضا بنسبة (98 ٪) P. قيمة العلاقة بين CMV IgG وعمر المريض هو (0.03) و قيمة العلاقة بين الحالة الاجتماعية للمرضى وتركيز (0.09) IgG

الخلاصة؛ هناك علاقة عالية بين CMV IgG وعمر المريض والوضع الاجتماعي للمرضى وتركيز IgG.

الكلمات الرئيسية: CMV ، سرطان الثدي ، IgG ، وسط الخرطوم

CHAPTER ONE

INTRODUCTION

1.1. Background

The most common cancer among women globally was breast cancer. Numerous genetic and environmental factors may play a role in the development of breast cancer [1]. Human cytomegalovirus (HCMV) is a widespread opportunistic *herpesvirus* that causes severe and fatal diseases in immunocompromised individuals including those who are organ transplant recipients, HIV-infected and patients with cancer. In vitro, HCMV can transform cells and deregulate other pathways relevant to *adenocarcinoma* pathogenesis, especially those affecting the cell cycle, mutagenesis, apoptosis, and angiogenesis when it has an oncomodulator role [2]. Breast cancer is the most common malignancy affecting females worldwide but conventional risk factors are able to explain only a small proportion of these cases. A possible viral etiology for breast cancer has been proposed and Epstein - Barr virus (CMV) is a widely researched candidate virus [3].

Cytomegalovirus (CMV) (from the Greek cyto-, "cell", and megalo-, "large") is a genus of viruses in the order *Herpesvirales*, in the family *Herpesviridae*, in the subfamily *Betaherpesvirinae* [1]. Humans and monkeys serve as natural hosts. There are currently eight species in this genus including the type species, human cytomegalovirus (HCMV, human *herpesvirus* 5, HHV-5), which is the species that infects humans. Diseases associated with HHV-5 include mononucleosis, and pneumonia. In the medical literature,

most mentions of CMV without further specification refer implicitly to human CMV. Human CMV is the most studied of all *cytomegaloviruses* [4].

Human cytomegalovirus is a species of the virus genus Cytomegalovirus, which in turn is a member of the viral family known as *Herpesviridae* or *herpesviruses* [5]. It is typically abbreviated as HCMV or, commonly but more ambiguously, as CMV. It is also known as human herpesvirus-5 (HHV-5). Within *Herpesviridae*, HCMV belongs to the Betaherpesvirinae subfamily, which also includes *cytomegaloviruses* from other mammals [3].

Humancytomegalovirus (HCMV) is a widespread opportunistic *herpesvirus* that causes severe and fatal diseases in immunocompromised individuals including those who areorgan transplant recipients, HIV-infected and patients with cancer [1]. In vitro, HCMV can transform cells and deregulate other pathways relevant to *adenocarcinoma* pathogenesis, especially those affecting the cell cycle, mutagenesis, apoptosis, and angiogenesis when it has an oncomodulator role [3]. The purpose of this study was to determine and investigate a possible association between progression of breast cancer and HCMV infection in our tumor collective of advanced breast carcinomas.

1.2. Rationale

Breast cancer is common in Sudan. Usually women present late in disease due to lack of efficient education, efficient health care system and low socio economic status.

Few publish data in Sudan that correlate CMV with breast cancer.

1.3. Objectives

1.3.1 General Objective

To detect association between CMV IgG concentration and breast cancer among Sudanese ladies by using the technique (ELFA) Enzyme Linked Fluorescent Assay in Center of Khartoum.

1.3.2 Specific Objectives

1- To detect cmv IgG in breast cancer patients.

2-To correlate between CMV I-gG concentration in breast cancer and patients age and control.

3-To correlate between CMV IgG concentration in breast cancer and patients social status and control.

CHAPTER TWO

LITERATURE REVIEW

2. Literature Review

Human *cytomegalovirus* CMV may be associated with many cancers in human. However, the role of CMV infection in breast cancer remains unclear.

2.1Cytomegalovirus (CMV)

Human cytomegalovirus is a species of the virus genus Cytomegalovirus, which in turn is a member of the viral family known as *Herpesviridae* or *herpesviruses* [1]. It is typically abbreviated as HCMV or, commonly but more ambiguously, as CMV. It is also known as human herpesvirus-5 (HHV-5). Within *Herpesviridae*, HCMV belongs to the *Betaherpesvirinae* subfamily, which also includes *cytomegaloviruses* from other mammals [3].

2.2Causes of CMV

Acquired cytomegalovirus can spread between people through bodily fluids such as saliva, semen, blood, urine, vaginal fluids, and breast milk. Infection may also occur by touching a surface infected with saliva or urine, and then touching the inside of the nose or mouth [5]. Most humans become infected during childhood, at daycare centers, nurseries, and places where children are in close contact with each other. However, by this age, the child's immune system is normally able to deal with an infection [6]. Recurring CMV can occur in patients with a weakened immune system due to HIV, organ transplantation, chemotherapy or taking oral steroids for over 3 months. Congenital CMV normally occurs when a woman is infected with CMV for the first time, either during her pregnancy or shortly before conceiving. Occasionally, a dormant CMV infection may recur during pregnancy, especially if the mother has a weakened immune system [7].

2.3 Types of CMV:

CMV is generally not a problem, except when it affects an unborn child or a person with a weak immune system, such as a recent transplant recipient or a person with human immunodeficiency virus, or HIV. In people with HIV, CMV infection can lead to organ failure, eye damage, and blindness. Improvement in antiviral medication has reduced the risk in recent years. Organ and bone marrow transplant recipients have to take immunosuppressants to lower their immune systems, so that their bodies do not reject the new organs [3]. Dormant CMV can become active in these patients and lead to organ damage. Transplant recipients may receive antiviral medicines as a precaution against CMV. During pregnancy, CMV infection can pass from the mother to the fetus. This is called congenital CMV. There are three main types of CMV infections: acquired, recurring, or congenital .Acquired, or primary, CMV is a first-time infection. Recurring CMV is when the patient is already infected. The virus is dormant and then becomes active due to a weak immune system [7]. Congenital CMV is when infection occurs during pregnancy and affects the unborn child.According to the CDC, around 1 in 150 newborns are already infected with CMV at birth. Most of these infants will have no signs or symptoms, but around 20 percent of them will have symptoms or long-term health complications, including learning difficulties. The symptoms may be severe, and they include vision and hearing loss, vision loss, small head size, weakness and difficulty using muscles, problems of coordination, and seizures [8].

2.4 Symptoms of CMV

The symptoms will depend on the type of CMV [9].

2.4.1 Acquired CMV

Most people with acquired CMV have no noticeable symptoms, but if symptoms do occur, they may include: fever, night sweats, tiredness and uneasiness, sore throat, swollen glands, joint and muscle pain and low appetite and weight loss [8].

2.4.2 Recurring CMV

Symptoms of recurring CMV vary, depending on which organs are affected. Areas likely to be affected are the eyes, lungs, or digestive system [7]. Symptoms may include: fever, diarrhea, gastrointestinal ulcerations, and gastrointestinal bleeding, shortness of breath, pneumonia with hypoxemia, or low blood oxygen, mouth ulcers that can be large, problems with vision, including floaters, blind spots, and blurred vision, hepatitis, or inflamed liver, with prolonged fever, encephalitis, or inflammation of the brain, leading to behavioral changes, seizures, and even coma [9].

2.4.3 Congenital CMV

Around 90 percent of babies born with CMV have no symptoms, but 10 percent to 15 percent of them will develop hearing loss, normally during their first 6 months of life [10]. The severity ranges from slight to total hearing loss. In half of these children, just one ear will be affected, but the rest will have hearing loss in both ears. Hearing loss in both ears can lead to a higher risk of speech and communication problems later on. If there are symptoms of congenital CMV at birth, they may include: jaundice, pneumonia, red spots under the skin, Purple skin splotches, a rash, or both, enlarged liver, enlarged spleen, low birth weight and seizures. Some of these symptoms are treatable [11].

2.5. Diagnosis of CMV

A blood test can detect the antibodies which are created when the immune system responds to the presence of CMV. A pregnant woman has a very small risk of reactivation infecting her developing baby [10]. If infection is suspected, she may consider amniocentesis, which involves extracting a sample of amniotic fluid to find out whether the virus is present. If congenital CMV is suspected, the baby must be tested within the first 3 weeks of life. Testing later than 3 weeks will not be conclusive for congenital CMV, because the infection could have happened after birth. Any patient with a weakened immune system should be tested, even if there is no active CMV infection [7]. Regular monitoring for CMV complications will include testing for vision and hearing problems [8].

2.6. Treatment of CMV

Scientists have been searching for a CMV vaccine, but as yet there is no cure. People with acquired CMV, who are infected for the first time, can use over-the-counter (OTC) painkillers such as Tylenol (acetaminophen), ibuprofen, or aspirin to relieve symptoms, and should drink plenty of fluids [12]. Patients with congenital or recurring CMV can use anti-viral medications such as ganciclovir to slow the spread of the virus. These medications may have adverse effects [6]. If there is extensive organ damage, hospitalization may be necessary. Newborns may need to stay in the hospital until their organ functions return to normal [12].

2.7. Complications of CMV

Healthy people very rarely become significantly sick from CMV infection. People with a weakened immune system, however, may develop CMV mononucleosis, a condition in which there are too many white blood cells with a single nucleus [13]. Symptoms include sore throat, swollen glands, swollen tonsils, tiredness, and nausea. It can cause liver inflammation, or hepatitis, and spleen enlargement. CMV mononucleosis is similar to classic mononucleosis, caused by the Epstein - Barr virus. EBV mononucleosis is also known as glandular fever [14].

Other complications of CMV are: gastrointestinal problems, including diarrhea, fever, abdominal pain, colon inflammation, and blood the in feces, liver function problems, central nervous system (CNS) complications, such as encephalitis, or inflammation of the brain and *pneumonitis*, or inflammation of lung tissue [11].

2.7 Prevention

The following precautions may help lower the risk of contracting CMV: Wash the hands regularly with soap and water. Avoid kissing a young child, including tear and saliva contact. Avoid sharing glasses and kitchen utensils, for example, passing round a drink [5]. Dispose of diapers, paper handkerchiefs, and similar items carefully. Use a condom to prevent the spread of CMV via vaginal fluids and semen.The CDC urges parents and caregivers of children with CMV to seek treatment as early as possible, whether it is medication or attending services such as hearing checks [8].

2.9. CMV and Breast cancer

Human cytomegalovirus (HCMV) is a widespread opportunistic *herpesvirus* that causes severe and fatal diseases in *immunocompromised* individuals including those who are organ transplant recipients, HIV-infected and patients with cancer [10]. In vitro, HCMV can transform cells and deregulate

other pathways relevant to *adenocarcinoma* pathogenesis, especially those affecting the cell cycle, mutagenesis, apoptosis, and angiogenesis when it has an oncomodulator role. Breast cancer is the most common malignancy affecting females worldwide but conventional risk factors are able to explain only a small proportion of these cases. A possible viral etiology for breast

cancer has been proposed and Epstein - Barr virus (CMV) is a widely researched candidate virus [13]. The purpose of this study was to determine and investigate a possible association between progression of breast cancer and HCMV infection in our tumor collective of advanced breast carcinomas[10].

2.10. Breast Cancer

Breast cancer is a disease that occurs when cells in breast tissue change (or mutate) and keep reproducing. These abnormal cells usually cluster together to form a tumor [14]. A tumor is cancerous (or malignant) when these abnormal cells invade other parts of the breast or when they spread (or metastasize) to other areas of the body through the bloodstream or lymphatic system, a network of vessels and nodes in the body that plays a role in fighting infection [15]. Breast cancer usually starts in the milk-producing glands of the breast (called lobules) or the tube-shaped ducts that carry milk from the lobules to the nipple. Less often, cancer begins in the fatty and fibrous connective tissue of the breast [16]. New cases of breast cancer are about 100 times more common in women than in men, but yes, men can get breast cancer too. Male breast cancer is rare, but anyone with breast tissue can develop breast cancer [17].

2.11. Causes of Breast Cancer

After puberty, a woman's breast consists of fat, connective tissue, and thousands of lobules, tiny glands that produce milk for breast-feeding. Tiny tubes, or ducts, carry the milk toward the nipple. In cancer, the body's cells multiply uncontrollably [15]. It is the excessive cell growth that causes cancer. Breast cancer usually starts in the inner lining of milk ducts or the lobules that supply them with milk. From there, it can spread to other parts of the body [18].

CHAPTER THREE

MATERIALS & METHODS

3.1. Study Design:

This study is an Analytical case control study.

3.2. Study Area:

This study was performed in Centre- Khartoum clinic.

3.3. Study Duration:

This study was performed during period from October 2017 to June 2018.

3.4. Study Population:

Sudanese patient with breast cancer.

3.5. Sample Size:

Fiftycase serum sample and fifty control serum sample were selected.

3.6. Sampling Technique:

Simple random sampling technique was used to collect samples.

3.7. Inclusion Criteria:

All patients with breast cancer.

3.8. Exclusion Criteria:

All ladies with breast cancer between (20 – 70) yare.

3.9. Data Collection:

The data was collected from Patient & co- Patient. Samples obtained from patients from canter Sudan.

3.10. Data Analysis:

Data were analyzed using statistical package for social sciences (SSPS) computer program version 21.0.

3.11. Ethical Considerations:

An ethical permission was obtained from relevant authorities. Samples were collected after agreement with laboratory administrations and Patient & co-Patient.

3.12. Controls:

Positive and negative control sections were used to evaluate the working solutions and to evaluate the testing kits. All Precautions and quality issues were be issued as manufacture instructions.

3.13. Principle:

The assay principle combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

After dilution, the sample is incubated with the SPR. Anti-CMV IgG antibodies present in the specimen will bind to the CMV antigen coating the interior of the SPR. Unbound components are eliminated during the washing steps.

During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antibodies present in the sample.

At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

3.14. Thresholds and interpretation of results:

Value (aU/ml)	Interpretation
< 4	Negative
from ≥4 to < 6	Equivocal
≥ 6	Positive
Value (aU/ml)	Interpretation

CHAPTER FOUR

RESULTS

4. Results

A total of 50 cases (patients with confirmed breast cancer) and 50 controls were included in this study. The age of patients was ranged from 30-70 years with average mean of 42.5 years.

From all subject 49 (98%) cases represented positive result for IgG CMV.

The ages were divided into four age groups the first category of 30- 40 years was represented 20 cases (40%) the second group 41-50 years was represented 14cases (28%) the third group 51-60 years was represented 12cases (24%) the last group 61-70 years was represented 3cases (6%) as indicated in figure 4.1.

This study involved the social status and represented 38 cases were married and 12 cases were single

Age mean= (42.56±10.34)

STD=10.34

Maximum=72

Minimum=28

Table (4.1): Association between study group and IgG concentration

Variables	IgG concentration		Total	P.value
	Positive	Negative		
Case	49	1	50	0.157
Control	48	2	50	
Total	97	3	100	

Table (4.2): Association between age groups of patients & control and IgG concentration

Association between age groups of patients and IgG concentration

Variables	Age groups				Total	P.value
	30-40	41-50	51-60	61-70		
Positive	20	14	12	3	49	0.031
Negative	0	0	0	0	0	
Total	20	14	12	3	49	

Association between age groups of control and IgG concentration

Variables	Age groups				Total	
	30-40	41-50	51-60	61-70		
Positive	16	17	10	5	48	0.052
Negative	2	0	0	0	2	
Total	18	17	10	5	50	

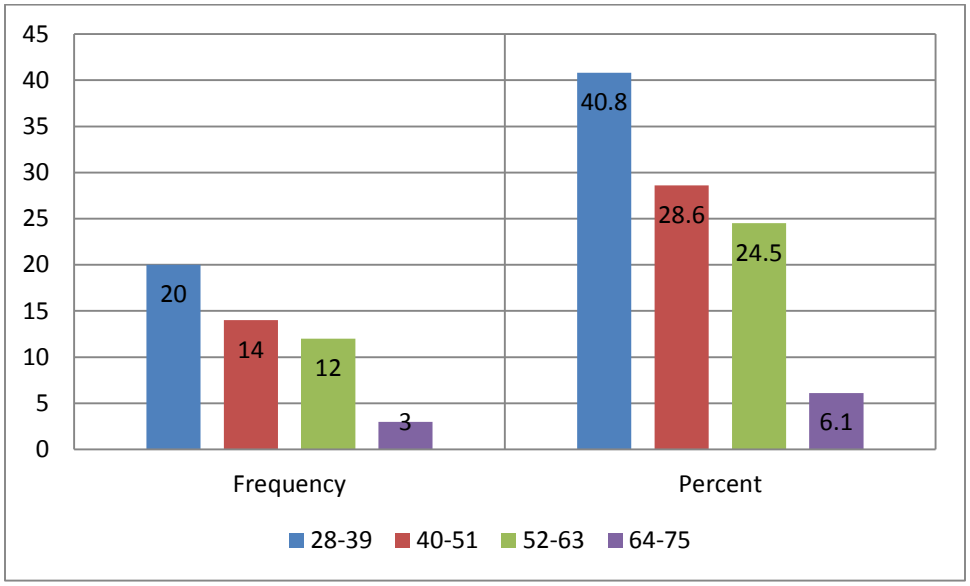

Fig 1: Frequency of age group among case group

Table (4.3): Association between social status of patients & control and IgG concentration

Association between social status of patients and IgG concentration				
Variables	**IgG concentration**		**Total**	**P.value**
	Married	**Single**		
Positive	38	11	49	0.09
Negative	0	0	0	
Total	38	11	49	

Association between social status of control and IgG concentration				
Variables	**IgG concentration**		**Total**	
	Married	**Single**		
Positive	43	5	48	0.06
Negative	2	0	2	
Total	45	5	50	

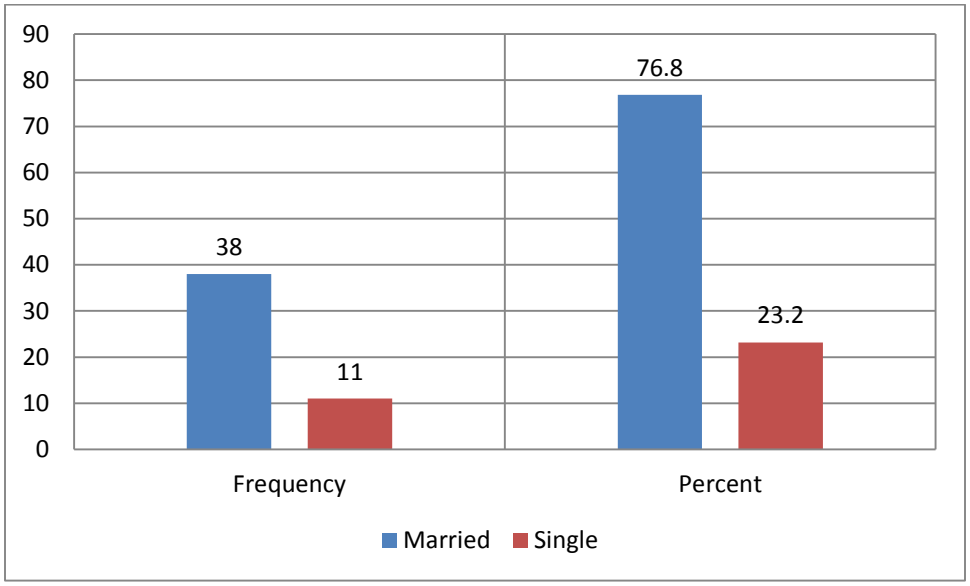

Fig 2: Frequency of social status among case group

CHAPTER FIVE

DISCUSSION, CONCLUSION & RECOMMENDATIONS

5.1Discussion

Human cytomegalovirus CMV may be associated with many cancers in human. However, the role of CMV infection in breast cancer remains unclear. In this study the mean age of cases was (42.56±10.34) years the reason for this age is the age of hormonal activity agreed with [19] and disagreed with [20] and [21]. Found mean age of CMV in women was 40 years while [20] reveled the mean age of occurrence for CMV is 52 years and [21] who reported the mean age is 32years by use ELFA technique.

In this study 49 (98%) cases represented positive result for IgG CMV in patients with breast cancer agreed with [19], [22] and [23] . [19] Who was found that IgG CMV positive in (57%) of patients with breast cancer [22] Who found elevation of CMV IgG levels in serum precedes the development of breast cancer. [23] Reported CMV IgG was positive in 70% of patients with breast cancer by using PCR technique.

There is significant relation between CMV IgG levels and patient's age ($p=0.031$) in this study according to hormonal activity which that is agreed with [24] and disagreed with [25]. Who was observed CMV higher 2:3 at younger age than older $p<0.001$. [25] Who was found no relation between CMV IgG levels and patient's age ($p=0.8$).

There is significant relation between social status of patients and IgG concentration in this study.

5.2 Conclusion

About 98% Present with represented positive result for IgG CMV.

There is significant relation between CMV IgG levels and patient's age.

There is significant relation between social status of patients and IgG concentration

5.3 Recommendation

1. Future studies to detect CMV IgM antibody.
2. More studies with large sample size and other biomarker should be carried.
3. Studies with advanced technique should be carried.

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