University of Babylon

College of Medicine

A Relationship Between Cytomegalovirus Infection and Incidence of Peptic Ulcer Disease

A Thesis

Submitted to the Council of the College of Medicine in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Microbiology

**By **

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جامعة بابل

كلية الطب

العلاقة بين الإصابة بفايروس *Cytomegalovirus* وحدوث مرض القرحة المعدية

رسالة مقدمة إلى مجلس كلية الطب- جامعة بابل وهي جزء من متطلبات نيل درجة الماجستير في الأحياء المجهرية الطبية

من قبل

نور سامي عبود الهادي

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بسم الله الرحمن الرحيم

((قل لو كان البحر مدادا لكلمات ربي لنفد البحر قبل أن تنفد كلمات ربي ولو جئنا بمثله مددا))

صدق الله العلي العظيم

سورة الكهف

الآيـة (١٠٩)

We certify that this thesis was prepared under our supervision at the University of Babylon as partial Fulfillment of the requirement for the degree of M.Sc. in Medical Microbiology….

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Dedication

To

My Beloved Parents

&

My Husband Ahmed

and My Son Mohammed

العلاقة بين الإصابة بفايروس *Cytomegalovirus*

وحدوث مرض

القرحة المعدية

أجريت هذه الدراسة في وحدي الناظور في مستشفى مرجان مستشفى الحلة التعليمي في محافظةِ بابل ومستشفى الكرامة التعليمي في محافظةِ بغداد للفترة من تشرين الثّاني ٢٠٠٤ إلى تموزٍ ٢٠٠٥. شملت هذه الدراسة ١٠٠ مريضا من مرضى القرحة المعدية و٥٠ شخصا ادخلوا كمجموعة سيطرة تم اختيارهم بصورة عشوائية من محافظة بابل لدراسة علاقة CMV في الإصابة بالقرحة المعدية. أعمار المرضى كَانتْ مِنْ ١٦ إلى ٧٠ سنة. متوسط العمر للمرضى كَان بعمر ٣٨ سنةً.اجري لجميع مرضى الدراسة فحص تنظير الجزء العلوي من الجهاز الهضمي وآخذت عينات الدم من كل شخص منهم بينما آخذت الخزع من ٣٨ مريض فقط.

عينات الدم التي تم جمعها اختبرت بواسطة فحص ELISA للكشف عن وجود أضداد CMV IgG and IgM. أما الخزع فقد فحصت نسيجيا بواسطة(صبغة الهيماتوكسلين-ايوسين) لتحديد الخلايا العملاقة واعتماد طريقة التألق للكشف أو التعرف على(الجسم الضمين) داخل النواة وهي صفة خاصة ببروتين CMV .

أظهرت الدراسة بأن جميع مرضى القرحة المعدية يمتلكون أضداد CMV IgG بينما ٦ أشخاص فقط من ٥٠ شخص ِسيطرة طبيعي يمتلكون أضداد CMV IgG. و أحد عشر مصاب بالقرحة المعدية من ١٠٠ شخص وجدوا بأنهم يمتلكون أضداد CMV IgM بينما جميع أشخاص السيطرة لا يمتلكون أضداد CMV IgM.

لقد وجد فرق معنوي كبير في عدد المصابين ب CMV بشكل مزمن بين الفئات العمرية المختلفة، أعلى إصابة كانت بين ٣٠-٤٠ سنة بينما أعلى إصابة ب CMV بشكل حاد وجدت في المجموعتين العمريتين (٢١-٣٠) و (٤١-٥٠) سنة .

هناك فرق معنوي في عدد المصابين بالقرحة المعدية من الذكور والإناث، حيث وجدت نسبة الذكور إلى الإناث هي ٣ :١ .

هناك فرق معنوي في عدد المصابين بالقرحة الهضمية في منطقتي الريف والمدينة حيث وجدت النسبة بين المصابين في الريف إلى المصابين في المدينة ٢:١ .

الفحص النسيجي (صبغة الهيماتوكسلين-ايوسين) اظهر الخلايا العملاقة ل CMV في جميع الخزع المأخوذة من المصابين بالقرحة الهضمية. أظهرت تقنية التألق كشفت عن وجود المستضد الخاص ل CMV في النسيج المعدي في كافة الخزع المأخوذة.

Abstract

This study was conducted in the endoscopies units of Merjan Hospital, Babylon Teaching Hospital, Hilla City and Al- Karama Teaching Hospital in Baghdad City during November ٢٠٠٤ to July ٢٠٠٥. A hundred patients with peptic ulcer and ٥٠ healthy people as a control group were selected randomly to study the role of CMV in occurrence of peptic ulcer . The ages of patients were between (١٦- ٧٠) years old. The mean age was ٣٨ years old. Upper Gastro-intestinal tract endoscopy was done for all patients in this study. Blood sample was taken from each patient, and biopsies were taken from ٣٨ patients only .

Blood samples obtained from all study groups were tested by ELISA for detection of anti CMV IgG and IgM antibodies. Biopsies were used in histological examination (hematoxylin – eosin stain) to detect giant cell formation and fluorescent technique was adopted to diagnose or identify specific viral (intranuclear) inclusion which were specific and characteristic of CMV proteins.

The study shows that all peptic ulcer patients have had positive anti CMV IgG antibody, whereas only ٦ out of ٥٠ normal people have had positive anti CMV IgG antibody. Only ١١ peptic ulcer patients out of ١٠٠ patients have had positive anti CMV IgM antibody, whereas no one from the control group people have shown anti CMV IgM antibody. The highest occurrence of CMV (chronic

state) was in the age of ٣١ to ٤٠ years old. The difference between age groups were highly significant (P < ٠.٠١). The highest occurrence of CMV (acute state) was in the age of ٢١-٣٠ and ٤١-٥٠ years old. There was a significant difference (P< ٠.٠١) between male and female in peptic ulcer disease. The males to females ratio were ٣:١. There was a significant difference (P< ٠.٠١) between rural and urban area in peptic ulcer disease. The ratio of rural to urban peptic ulcer patients was ١: ٢.

The histologic examination (hematoxylin – eosin stain) of biopsies collected from peptic ulcer patients showed CMV giant cell formation in all biopsies. Fluorescent technique showed the presence of CMV specific antigen in the gastric tissue in all biopsies too.

ACKNOWLEDGEMENTS

I would like to express my thanks to Allah the Most Gracious and Most Merciful.

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Decision of discussion committee

We the examining committee, after reading this thesis and examining the student in its contents, find it adequate as a thesis for the degree of Master of Science in Microbiology.

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Thesis Contents

List of Tables

List of Figures

List of Abbreviations

Symbol	Description
Abs	Antibodies
CDC	Center for disease control and prevention
CFT	Complement fixation technique
CMV	Cytomegalovirus
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
EM	Electronic microscope
gB	Glycoprotein

GIT	Gastro intestinal tract
HCMV	Human cytomegalovirus
HRP	Horseradish peroxidase
IFT	Indirect fluorescent technique
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Iu /ml	International unit per milliliter
Meq/l	Millie equivalent per liter
mRNA	Messenger ribonucleic acid
$NSAID_S$	Non-steroidal anti-inflammatory drugs
Symbol	Description
O.D.	Optical density
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
pp٦٥,pp٦٧	Phosphoprotein ٦٥, Phosphoprotein ٦٧
RNA	Ribonucleic acid
r.p.m.	Round per minute
TMB	Tetramethylbenzidin

Conclusions

And

Recommendations

Conclusions

١. All peptic ulcer patients have had positive anti CMV IgG antibody, whereas only ٦ out of ٥٠ normal people have had positive anti CMV IgG antibody.

٢. Only ١١ peptic ulcer patients out of ١٠٠ patients have had positive anti CMV IgM antibody, whereas all ٥٠ normal people have had negative anti CMV IgM antibody.

٣. The highest incidence of CMV (chronic state) occurs in the age of ٣١ to ٤٠ years old. The difference between age groups were highly significant.

٤. The highest incidence of CMV (acute state) occured in the age of ٢١-٣٠ and ٤١-٥٠ years old.

٥. There was a significant difference between males and females in peptic ulcer disease occurrence. The male to female ratio was ٣:١.

٦. There was a significant difference between rural and urban areas in peptic ulcer disease occurrence.

٧. The histological examination (hematoxylin – eosin stain) of biopsies collected from peptic ulcer patients showed CMV giant cell formation.

٨. Fluorescent technique showed the presence of CMV specific antigen in the stomach tissue.

Recommendations

This study recommends:

١. Avoiding CMV- infected blood. Since most blood banks do not care about routine tests for CMV, I suggest adopting CMV test as a routine test in all blood banks

٢. Washing hands with soap and water frequently.

٣. Not sharing drinking cups, eating utensils, cigarettes pipes, food like ice cream cones, etc.

٤. Adopting antiviral chemotherapeutics drug to treat peptic ulcer patients which will possibly help in the treatment of peptic ulcer patients and decrease the period of ailment.

٥. Use ELISA technique because it proved to be simple and easy to detect the anti CMV IgG and IgM antibodies.

٦. I would like to recommend to study the relationship between bacterial cause example *Helicobacter pylori* and incidence of CMV infection.

Chapter One

Introduction

Chapter One

Introduction

A peptic ulcer is a lesion on the lining of the stomach or duodenum, which is the beginning of the small intestine (Graham, *et. al.,* ١٩٩٩). The disease has relatively low mortality, but it results in substantial human suffering and high economic costs (Bujanda, ٢٠٠٠).

In the early ٢٠th century, the pathogenesis of the disorder was believed to be related to stress and dietary factors (Han, ٢٠٠٢). Chronic peptic ulcer is a major problem of modern society (Penston and Wormsley, ١٩٩٢). The disease may affect people between (٢٠ to ٦٠) years of age with the highest incidence between (٣٠ to ٥٠) years of age .Wise like men are affected more than women (Loginov *et. al.,* ١٩٩٨). Diagnosis is best made by upper gastrointestinal tract endoscopy, and in about ٩٠٪ of patients the ulcer is confined in the first part of the duodenum, within two centimeters of the pylorus (Lawrence, ١٩٨١).

Peptic ulcer disease is a chronic inflammatory condition of the stomach and/or duodenum that affects as many as ١٠ percent of people in the United States at some time in their lives (Bujanda, ٢٠٠٠). It has been reported that nearly ٤٠ ٠٠٠ peptic ulcer patients were admitted to hospital every year in the United Kingdom, while more than ٤٥٠٠ people die from the disease annually (Pounder, ١٩٩٠). The annual death rate from peptic ulcer has steadily increased

since ١٩٧٠, especially among women and old people (over ٧٤ years) (Dong *et. al.,* ٢٠٠٤). Smoking, stress, diet and familial susceptibility are the main predisposing factors for the occurrence of peptic ulcer. All these factors can increase peptic activity and result in hypersecretion of gastric acid (Balslev, ١٩٨٨; Khuro, ١٩٨٩).

Economic embargo imposed on Iraq from ١٩٩٠ to ٢٠٠٣ had a significant effect on these factors, particularly stress, diet, and economic status and increased the chances of occurrence of peptic ulcer and probably accentuated its course resulting in a higher rate of complications (Al-Jawher, ١٩٩٧). A bacterial infection (*Helicobacter pylori)* were claimed to be one causative agent but some ulcers were caused by long-term use of nonsteroidal anti-inflammatory agents (NSAIDs), like aspirin and ibuprofen (McManus, ٢٠٠٠).

Alternative causes of gastric ulcer should be investigated. Indeed, CMV is an opportunistic virus characterized by latent infection and may thus appear in previously damaged tissues or under different predisposing factors. However, a published recent studies have suggested that CMV, rather than *Helicobacter pylori,* may be the main inducing or activating (causative) pathogen of peptic ulcer patients (Chiu *et. al.,* ٢٠٠٤). Histopathologic findings and cultures were negative for *Helicobacter pylori.* Moreover, CMV inclusion bodies observed on initial pathological findings clearly

indicated the existence of a CMV disease at the patient's admission (Bruno *et. al.,* ٢٠٠٥).

Cytomegalovirus (CMV) infections are common and usually asymptomatic in otherwise healthy children and adults, however the incidents and spectrum of disease in new born and in immunocompromised hosts established this virus as an important human pathogen (Murray *et. al.*, ٢٠٠٣). CMV has a worldwide distribution and infects humans of all ages, with no seasonal or ecological pattern of transmission (Mandell *et. al.*, ٢٠٠٠). CMV is found universally throughout all geographic locations and socioeconomic groups, and infects between ٥٠٪ and ٨٥٪ of adults in the United States by the age of ٤٠ years. CMV is also the virus most frequently transmitted to a developing child before birth (Murray *et. al.*, ٢٠٠٣). CMV is more wide spread in developing countries and in areas of lower socioeconomic conditions. For most healthy individuals who acquire CMV after birth, there are few symptoms and no long term health consequences (CDC, ١٩٩٧).

CMV induced intestinal perforations are hard to diagnose and may be observed throughout the gastrointestinal tract. Isolated stomach perforation is exceptional (Bruno *et. al.*, ٢٠٠٥).

In Iraq, a little information is available about the relation between peptic ulcer and CMV, so this study aimed at:-

١-Investigating the relationship between peptic ulcer and Cytomegalovirus infection taking into consideration the presence of the following parameters.

a. Detection of IgG and IgM anti-CMV antibody and comparing that between infected patients and normal control group.

b. The presence or identification of giant cells formation on biopsy specimens, which is a diagnostic feature for herpes virus that is characterized by latent infection and especially CMV which affects mostly the digestive system.

c. The presence of (intranuclear) inclusion body which can be detected by fluorescent technique and its characteristic of CMV.

٢-On the bases of the above parameters, proving that CMV might play a causative role for peptic ulcer formation which then may be colonized by other blamed organism (*Helicobacter pylori)*.

Chapter Two

Literatures review

Chapter Two

Literatures review

٢-١ Digestive System

The digestive tract can be divided into five parts mouth, pharynx, esophagus, stomach, small and large intestine (Steven and James, ١٩٩٧). The digestive system from the mouth to the end of the colon is

responsible for all physical and chemical processes that occur during and after eating food.
The epithelial tissue that lines this muscular tube differs in its composition according to its functions and location in the digestive tract (Ganong, ١٩٩٧). Some cells of this tract secrete different digestive materials including enzymes such as pepsinogen which works with the help of other secretions such as
hydrochloric acid in converting the nutrient to a simple form to be transferred by blood or lymph to different body tissues. These elements will be useful in metabolic process (Steven and James, ١٩٩٧).

٢-١-١ Anatomy and histology of stomach

The fully developed stomach is the largest dilatation of the gut and lies between the esophagus and the duodenum (David and James, ١٩٩٧). The size and shape of stomach are different among normal people (Ganong, ١٩٩٧). The growth and development of stomach in human is asymmetrical. The middle part and left side edge is called greater curvature, which starts, from left side of stomach connection to duodenum including the left upper part and lower edge of stomach. Lesser curvature is located on the other side. It is ٤-٥ times shorter than greater curvature and starts from right side of stomach connection with esophagus extending down forming concave part of stomach. It bends sharply forming incisura angularis (Anthony *et.al.*, ١٩٩٧).

The stomach is divided into five segments: the cardia, fundus, body, antrum, and pylorus, which are macroscopically similar but histologically distinct. (Figure ٢-١)

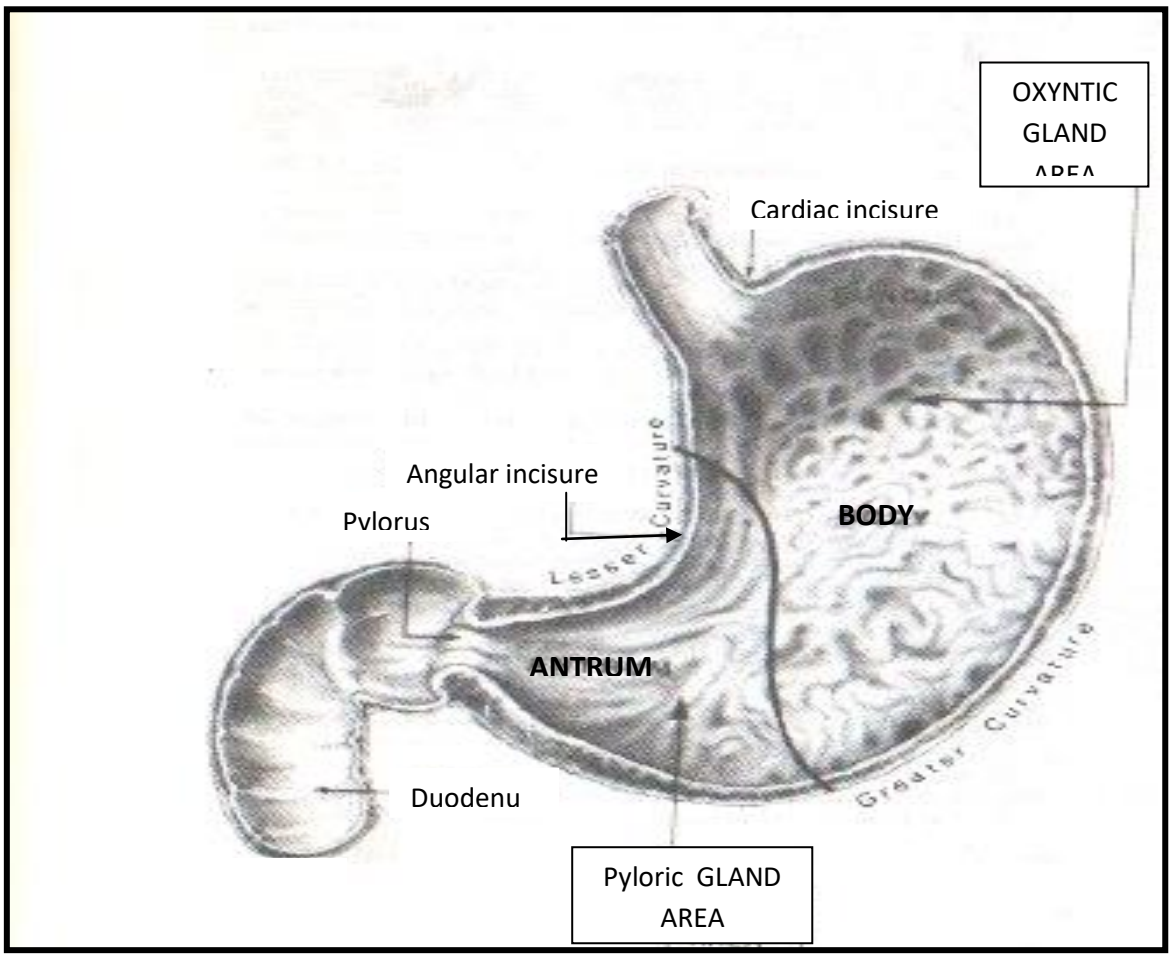


Figure ٢-١ The Parts of Stomach Anatomy (Lloyd and Samuel, ١٩٨٥)

Histologically the stomach is composed of four tissue layers: the mucosa, submucosa, muscularis propria, and serosa. The mucosa layer of fundus and body contains oxyntic (parietal) cells which manufactures and secrete hydrochloric acid and gastric intrinsic factor, chief cells (zymogen, peptic) cells which make and secrete pepsinogen, mucous cells which produce mucus. (Ganong, ١٩٩٧). Endocrine and paracrine cells, scattered throughout the glands of the proximal and distal stomach, have prominent secretory granules that contain peptide hormones, each cell usually contains only one peptide hormone. (Lloyd and Samuel, ١٩٨٥). The hormones gastrin produced by G cells in the antrum and somatostatin secreted from D cells throughout the stomach interact to modulate gastric secretion and motility. Gastrin stimulates whilst somatostatin suppresses acid secretion. (Christophe *et. al.*, ٢٠٠٢).

The mucus secreting cells predominate in the cardia while pepsinogen-secreting cells are rare (Eastwood, ١٩٨٩). All these cell

types form gastric glands; however, these glands represent most thickness of mucosa layer. The gastric glands are either simple tubular or compound tubular. They are opened on gastric pits and through these pits the secretion is released from these glands to gastric lumen (Steven and James, ١٩٩٧).

٢-١-٢ Anatomy and histology of duodenum

The small intestine and its glands are the most important parts of gasto-intestinal canal since the final digestive process of the meal is occurred in it as well as the whole operation (Guyton and John, ٢٠٠٠).

The small intestine can be divided into three main parts i.e. duodenum, jejunum and ileum (Anthony *et. al.,* ١٩٩٧).

The duodenum is the first part of the small intestine which extends from the pylorus about ٢٠ to ٣٠ cm. and ends at the ligament of Treitz, where the jejunum begins. Adhesive bands between the duodenal-jejunal junction and the retroperitoneum on the left side of the abdomen marks this region (David and James, ١٩٩٧). The duodenum is divided into four portions, although there are no sharp lines of demarcation between the different parts. The first portion extends from the pyloric sphincter to the apex of the duodenal bulb and is devoid of mucosal folds. It is characterized microscopically by submucosal Brunner's glands that secrete a clear viscous alkaline material (pH ٨-٩). Throughout the remainder of the duodenum, the mucosal folds are circular. The second portion or the descending limb of the duodenum contains the ampulla of vater, into which the common bile duct and pancreatic duct empty their contents. The third, transverse portion of the duodenum lies anterior to the spine and is crossed on its ventral surface by the superior mesenteric artery and vein. This is the portion of the duodenum most often ruptured by blunt trauma. The fourth portion of the duodenum is the ascending limb. It ends at the ligament of Treitz (Hardy *et. al.,* ١٩٨٨).

The structure of duodenum is an extension for stomach wall layers. The Same four layers of stomach are found here, the most important cells in duodenum structure is mucus cell, enterocytes, goblet cells, paneth's cells and endocrine cells (Guyton and John, ٢٠٠٠).

The function of the duodenum is regulated by vagus nerve, splanchnics nerve and enteric autonomic nervous system (Lloyd and Samuel, ١٩٨٥).

٢-١-٣ Gastric secretion

Stomach are lined with epithelial cell which secret gastric juice. The gastric juice mainly consists of five materials: - hydrochloric acid (HCl), enzymes such as (pepsinogen, amylase, lipase), intrinsic factor, electrolytes such as (HPO_4^+, Cl^-, Mg^{+2}, K^+, Na^+) and mucus. The average secretion of gastric juice is about ٣ liter/day in normal situation and it is under the effect of nerves, mechanical and hormonal control (Guyton and John, ٢٠٠٠).

٢-١-٤ Hydrochloric acid

Hydrochloric acid is actively secreted by the parietal cells and can be concentrated as high as ١٥٠ meq/L, a concentration of hydrogen ion one million time than that in the blood. Gastrin, histamine, and acetylcholine stimulate the parietal cell to secrete acid (Lloyd and Samuel, ١٩٨٥). The Hydrochloric acid kills many ingested bacteria, aids protein digestion, and provides the necessary pH for pepsin to start protein digestion (Ganong, ١٩٩٧).

٢-٢ Peptic Ulcer

A peptic ulcer is a sore on the lining of the stomach or duodenum, which is the beginning of the small intestine (Graham, *et. al.*, ١٩٩٩). If peptic ulcers are found in the stomach, they're called gastric ulcers. If they're found in the duodenum, they're called duodenal ulcers. A patient may have more than one ulcer (Lahaie and Gaudrean, ٢٠٠٠). About ٩٩٪ of peptic ulcers are either in the duodenum or stomach. The ratio is ٤:١ approximately (Jay, ١٩٩٤). Generally, gastric ulcer is either acute or chronic and recurrent (Brook, ١٩٨٥). Acute ulcer is a break or discontinuation of epithelial tissue lining of digestive canal and some time extends to the submucosa layer. It usually spreads in several locations and does not leave scar after healing (Hardy *et. al.*, ١٩٨٨). However the chronic and recurrent ulcer is usually single except about ٥ to ٢٠ % of cases where

they appeared in more than one location. It usually extends to submucosa layer and leaves scar or fibrosis after healing (Sleisenger and Fordtran, ١٩٩٧).

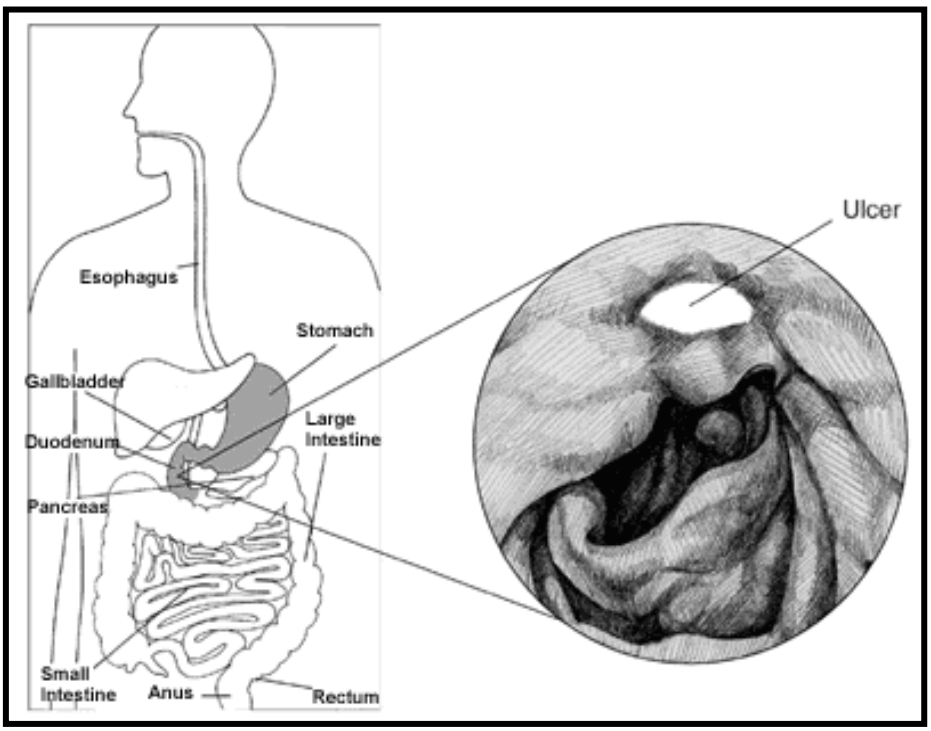
٢-٢-١ Incidence of peptic ulcer:

The number of peptic ulcer patients has increased during the last thirty years due to the developments in the world of different features of life and changing the style of living from rural to urban (Robbins, ٢٠٠٠). The occurrence of peptic ulcer is very rare before the age of ٢٠ years. The possibility of infection with peptic ulcer increases gradually as the age increases until it reaches the peak at the age of ٥٠ years for duodenal ulcer and after that for stomach ulcer (Jay, ١٩٩٤). A number of researchers mentioned that males suffered from peptic ulcer more than females but there was disagreement among them about the ratio of male to female infection. (Blumenthal, ١٩٦٨) pointed out that the ratio was ٣:١. This result was confirmed by Loginov *et. al.* (١٩٩٨) who came out with a ratio of ٣.٥:١, but Tovey *et. al.* (١٩٨٩) found that the ratio of male to female infection was ١٨:١.

The effect of smoking habit and its relation with peptic ulcer have been studied. Eastwood (١٩٨٩) found that there was a significant effect on development and continuity of peptic ulcer. While Unge and Friksoon (١٩٩٣) believe that smoking has no significant role on peptic ulcer infection but it will worsen the infection for the already present one and limit the effect of medicine.

٢-٢-٢ Distribution of peptic ulcer

Different scientists studied the effect of the geographical localities on peptic ulcer and they had different opinions. Robbins (٢٠٠٠) showed that there was a significant effect of geographical location on the peptic ulcer infection and the average of cases of peptic ulcer differed from one country to another and from location to location within the same country. This result has been observed by Sonnenberg (١٩٩٥) but he has added that there is a lack of information in this field and there is a need for more studies to get a



reliable result. People of urban areas suffer from peptic ulcer infection more than people of rural area. The kind of food may be behind that variation since the urban people feed mainly on preserved food, which might change to ulcerogens (Sleisenger and Fordtran, ١٩٩٧).(Figure٢-٢)

Figure ٢-٢ Peptic ulcer in the wall of the stomach and duodenum (Goodgame, ١٩٩٣)

٢-٣ The Causative Agents of Ulcer

One cause of peptic ulcer is bacterial infection, but some ulcers are caused by long-term use of nonsteroidal anti-inflammatory agents (NSAIDs), like aspirin and ibuprofen (Han, ٢٠٠٢). A published recent study has suggested that CMV may be the main inducing or activating (causative) pathogen of peptic ulcer patients (Chiu *et. al*., ٢٠٠٤).

٢-٣-١ *Helicobacter pylori (H. pylori)*

Helicobacter pylori is a type of bacteria. It is curved or spiral-shaped, Gram negative, strictly microaerophilic and motile by means of sheathed flagella. These properties represent the general characteristics of most mucus which are associated with intestinal bacteria (Leslie *et. al.,* ١٩٩٨). Many researchers believe that *H. pylori* is responsible for the majority of peptic ulcers**.** *H. pylori* infection is common in the United States (Vaira *et. al.,*١٩٩٩).

Helicobacter pylori weakens the protective mucous coating of the stomach and duodenum, which allows acid to get through to the sensitive lining beneath. Both the acid and the bacteria irritate the lining and cause a sore, or ulcer**.** *H. pylori* is able to survive in stomach acid because it secretes enzymes that neutralize the acid. This mechanism allows *H. pylori* to make its way to the "safe" area--the protective mucous lining. Once

there, the bacterium's spiral shape helps it burrow through the lining (Lahaie and Gaudrean, ٢٠٠٠).

٢-٣-٢ Cytomegalovirus (CMV)

Cytomegalovirus is a member of the herpes virus group(Herpsviridae), which includes the viruses that cause chicken pox, mononucleosis ("mono") and herpes simplexes ١ and ٢. All viruses share the ability to remain dormant in the body for a long time (Nancy, ٢٠٠٣). It is found in all populations in all parts of the world. CMV infection is very common; between ٥٠ and ٨٥ percent of adult in the United States are infected with CMV by age ٤٠ (CDC, ١٩٩٧).CMV spreads from one person to another. Infection requires close, intimate contact with a person excreting the virus in their Saliva, semen, vaginal secretions, blood, urine, and breast milk.

In all geographical areas wied spread of HCMV infection involving all socioeconomic groups is observed; a significant most elevated seroprevalence in north african and asian ethnic groups as compared to Western populations is noticed; besides, HCMV is absolutely the virus most frequently transmitted in the perinatal period. In the immunocompetent host, the HCMV infection is symptomless in the great majority of cases; in the symptomatic cases it shows the clinical features of a self-limited mononucleosis-like syndrome (Foti *et. al.,* ٢٠٠٢). It is the most important infectious agent in transplant recipients. The critical step in the pathogenesis of CMV

infection is the reactivation of latent virus, which is activated by the immunosuppressive therapy and/or alloantigenic stimulation (Gondo, ١٩٩٨). Cytomegalovirus infections in the gastrointestinal tract of normal hosts are very unusual but a common cause of morbidity in immunocompromised hosts. We believe that Cytomegalovirus may have a role in the pathogenesis of gastrointestinal lesions in nonimmunocompromised patients (Arnar, ١٩٩١).

Cytomegalovirus (CMV) is a ubiquitous infectious agent, recognized as an important pathogen in patients from the neonatal period through adulthood. Although CMV infections in the gastrointestinal tract of immunocompromised individuals have been reported in increasing frequency in recent years, there have been only a few reports of CMV infections in the gastrointestinal tract of normal hosts **(**Chu *et. al.,* ١٩٩٤). However, gastritis is an infrequent manifestation of infection by Cytomegalovirus (CMV) in a healthy host (Cheung and Ng IO, ١٩٩٣). This complication is usually associated with a mononucleosis syndrome during the course of a disseminated infection. Macroscopically, it presents with edema and mucosal congestion, multiple erosions or ulcers. Histologic examination of the endoscopic biopsies allows the etiologic diagnosis to be established in most cases. In immunocompetent patients the clinical course of gastritis by CMV is usually self-limited (Vergara *et. al.,* ١٩٩٨).

Beekhuis and Karrenbeld (١٩٩٧) found Multiple fundic gastric ulcers without *Helicobacter pylori* and the patient did not take non-steroidal anti-inflammatory drugs (NSAIDs). The diagnosis was primary Cytomegalovirus infection, based on the demonstration of infected cells in the biopsy specimens, using specific monoclonal anti-Cytomegalovirus antibodies.

While only a few cases of Cytomegalovirus-associated gastric ulcer in non-immunocompromised hosts have been reported, this entity may be more frequently detected when careful histological examination is performed in the active stage rather than postponed until after healing of the ulcer. The upper gastrointestinal endoscopic study revealed multiple gastric ulcers. The endoscopic biopsy specimens obtained from٧٥-year-old woman with epigastric pain on the seventh day in hospital disclosed a few typical intranuclear cytomegalovirus inclusions.. No immunologic abnormalities were demonstrated by any laboratory tests (Yokose *et. al.,* ١٩٩٥) **.**

٢-٣-٢-١ History

In ١٩٠٤, Ribbert first identified histopathological evidence of CMV probably in tissues from a congenitally infected infant. Ribbert mistakenly assumed that the large inclusion-bearing cells he observed at autopsy were from protozoa (incorrectly named ***Entamoeba mortinatalium***). But in ١٩٢٠, Goodpasture correctly postulated the viral etiology of these inclusions. Goodpasture used

the term cytomegalia to refer to the enlarged, swollen nature of the infected cells (Goodpasture and Talbot, ١٩٢١). Human CMV (HCMV) was first isolated in tissue culture in ١٩٥٦, and the propensity of this virus to infect the salivary gland led to its initial designation as a salivary gland virus (Gleaves *et.al.,* ١٩٨٥).

In ١٩٦٠, Weller designated the virus Cytomegalovirus (Weller, ١٩٧١). A descriptive study published in ١٩٦٤ suggested that CMV may cause gastrointestinal disease in patients without detectable immunodeficiency (Levine *et. al.,* ١٩٦٤). Immunocompetent patients have developed gastrointestinal CMV disease associated with community-acquired, acute primary CMV infection (Diepersloot *et. al.,* ١٩٩٠), blood transfusions (Villar *et. al.,* ١٩٨٤), or sexual transmission (Rabinowitz *et. al.,* ١٩٨٨). During the ١٩٧٠s and ١٩٨٠s, knowledge of the role of CMV as an important pathogen with diverse clinical manifestations increased steadily. Several authors have reported (Lachman *et.al.,*١٩٧١; Leonidas *et.al.,*١٩٧٣; Stillman *et.al.,*١٩٨١; Marks *et.al.,*١٩٨٦) an acute, self-limited gastropathy in children, associated with marked hypertrophy of gastric folds and protein-losing enteropathy. These patients had either serologic, histologic, or culture evidence of CMV infection, or all three (Goodgame, ١٩٩٣).

Although enormous progress has recently been made in defining and characterizing the molecular biology, immunology, and antiviral

therapeutic targets for CMV, considerable work remains in devising strategies for prevention of CMV infection (Demmler, ١٩٩٦).

Of all the human herpesviruses described to date, Cytomegalovirus (CMV) is arguably the one whose infection causes the most morbidity and mortality. Although primary infection with this agent generally does not produce symptoms in healthy adults, several high-risk groups, including immunocompromised organ transplant recipients and individuals infected with HIV, are at risk of developing life- and sight-threatening CMV disease (Bobak, ٢٠٠٣) . In addition, CMV has emerged in recent years as the most important cause of congenital infection in the developed world, commonly leading to mental retardation and developmental disability (Schleiss and McVoy, ٢٠٠٤) .

٢-٣-٢-٢ Structure

Cytomegalovirus is a member of the family Herpsviridae. The name means “very big cell virus” which includes Epstein-Barr virus, herpes simplex virus type ١and ٢, Varicella-Zoster virus, and human herpes viruses ٦,٧, and ٨. It is classified in subfamily Betaherpesvirinae. Complete CMV particles have a diameter of ١٢٠ to ٢٠٠ nm and consist of a core containing double stranded DNA, an icosahedral capsid with ١٦٢ capsomeres, an amorphous tegument or matrix and surrounding phospholipid rich envelope. Electron microscopic features of CMV include virions morphologically indistinguishable from those of other herpes viruses, a high ratio of defective viral particles, and the presence of spherical particles called dense bodies (Ellen *et.al*, ٢٠٠٣). Human Cytomegalovirus (HCMV) is a ubiquitous pathogen that infects only humans (Jiping and Stinski, ٢٠٠٢). The capsids found in the cytoplasm of

infected fibroblasts by adapted strain human Cytomegalovirus (CMV) have bristle-like surface coating on them. This coating was claimed to be one of the important differences of CMV from herpes simplex virus. It is well known, that capsids of CMV increased in their over-all diameter through the process of moving from the nucleus to the cytoplasm. In some instances, however, uncoated capsids were detected in the cytoplasmic portions especially in the vicinity of tubular structures in the cytoplasm. It seemed that these uncoated capsids might be formed in the cytoplasm but had not emigrated from the nucleus (Kimura *et. al.*, ١٩٧٦) .

٢-٣-٢-٣ Replication

Cytomegalovirus (CMV) is generally described as a slowly replicating virus (Vincent *et. al.,*١٩٩٠) . It attaches to the cell surface at the site of the receptor for fibroblast growth factor. After entry into the cell the virion is uncoated and the genome DNA change its configuration from linear to circular. Early virus messenger RNA (mRNA) is transcribed by host cell DNA polymerase and then translated into early nonstructural protein in the cytoplasm. Two of these early proteins, thymidine kinase and DNA polymerase are important because they are sufficiently different from the corresponding cellular enzymes to be involved in the action of antiviral drugs, eg, acyclovir (Brooks *et. al.*, ٢٠٠٤).

Immediate early gene transcription occurs in the first ٤ hours following viral infection, when key regulatory proteins, which allow the virus to take control of cellular machinery, are made. The major

immediate early promoter of this region of the CMV genome is one of the most powerful eucaryotic promoters described in nature, and this has been exploited in modern biotechnology as a useful promoter for driving gene expression in gene therapy and vaccination studies (Jiping and Stinski, ٢٠٠٢).

Immediate early proteins are those whose synthesis is activated by a protein brought in by the incoming parental virion; no new viral protein synthesis is required for the production of the five "immediate early" protein (Brooks *et. al*, ٢٠٠٤) .

The early protein on the other hand do require the synthesis of new viral regulatory proteins to activate the transcription of their mRNA (Matthew and Compton, ٢٠٠٢) .

The viral DNA polymerase replicates the genome DNA, at which time early protein synthesis is shut off and late protein synthesis begins (Brooks *et. al*, ٢٠٠٤).

Finally, the late gene products are made approximately ٢٤ hours after infection, and these proteins are chiefly structural proteins that are involved in virion assembly and egress (Fentunato and Spector, ١٩٩٩).

Structural proteins are transported to the nucleus, where virion assembly occur. The virion obtains its envelope by budding through the nuclear membrane and exits the cell via tubules or vacuoles that

communicate with the exterior. In latently infected cells, multiple copies of Cytomegalovirus DNA are found in the cytoplasm of infected neurons. Only a few genes are transcribed and none are translated into protein (Brooks *et. al*, ٢٠٠٤). The complexity of the CMV replicating cycle is paralled by the structural intricacy of the virion. CMV has at least nine membarane-associated glycoprteins in its envelope (Kari *et. al.,* ١٩٩٠). Of these glycoprotein B (gB) is the most abundant and highly conserved glycoprotein among the herpesviruses. (gB) plays key roles in the process of CMV entry into host cells. This process is a multistep cascade beginning with attachment of the virus to the cell surface and ending with fusion of the virus envelope with the cell plasma membrane (Matthew and Compton, ٢٠٠٢). (Figure ٢-٣)

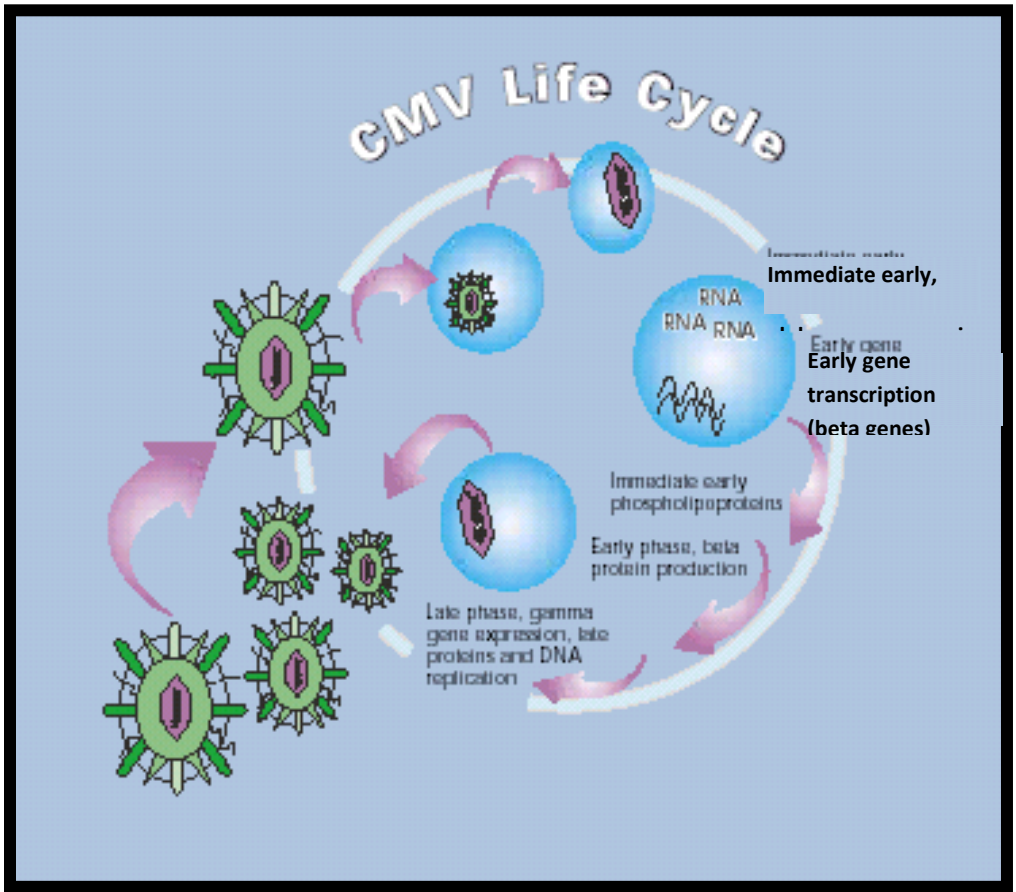
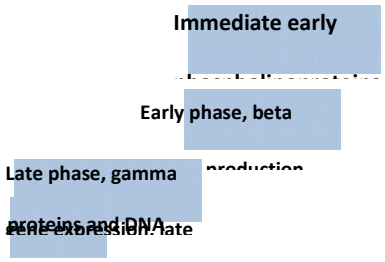


Figure ٢-٣ Replication Cycle of CMV (Erik, ٢٠٠١)

٢-٣-٢-٤ Transmission

Transmission most often occurs when these body fluids are in



contact with the hands, then they are absorbed through nose or mouth. People can also become infected with CMV through sexual intercourse, blood transfusions, and transplanted organs; in addition, babies can also become infected before or during birth, or through breast-feeding (Brooks *et. al.*, ٢٠٠٤) .

a-Saliva - It is probably the main route through which the virus is transmitted postnatally. This is likely to be the route through which the virus is transmitted amongst children (Foti *et. al.*,٢٠٠٢) .

b-Sexual transmission - sexual transmission is possible but not proven beyond doubt. CMV is found in semen and in the cervix. However oral-oral contact frequently occurs before intercourse which may well be the route through which the virus has been transmitted after intimate contact. However, susceptible

homosexuals run a particularly high risk of being infected. Rectal intercourse seems to be a risk factor (Hansen *et.al.*, ١٩٩٤) .

c-Blood transfusion - In the early ١٩٦٠s, a postperfusion syndrome was recognized in open heart surgery patients who received large quantities of blood which were contaminated with CMV. However transmission of CMV by blood is clearly a rare event as only ٣ - ٥٪ of blood taken from seropositive donors leads to infection of seronegative recipients. It has not been possible to determine which donors have a high risk of transmitting the virus. It is possible that CMV persists in a latent state in blood leukocytes, and CMV is occasionally reactivated on transfusion (Bobak, ٢٠٠٣).

d-Organ transplantation - Patients undergoing renal transplantation are particularly at risk. Seronegative recipients have a ٥٪ chance of acquiring primary infection from seronegative donors compared to a chance of ٧٠ - ٨٠٪ of acquiring primary infection from seropositive donors. It seems that CMV is transmitted via infected cells in the donor kidney (Limaye *et. al.*, ١٩٩٧).

٢-٣-٢-٥ Pathogenesis

Primary infection and latency

Most CMV infections are acquired either in the perinatal period and infancy or in adulthood through sexual contacts (Ho, ١٩٩٠). Infection of the fetus can cause cytomegalic inclusion disease

characterized by multinucleated giant cells with prominent intranuclear inclusions, many organs are affected and widespread congenital abnormalities result (Brooks *et. al*, ٢٠٠٤). Although congenital infections due to primary CMV infection in pregnancy are a cause of substantial morbidity and death (Demmler, ١٩٩١), most primary CMV infections in immunologically healthy adults are asymptomatic or are associated with a mild mononucleosis-like syndrome. Serious gastrointestinal disease due to primary infection is rare. All primary infections resolve and enter a state of latency in which live virus is sequestered in a nonreplicative state. Persons with latent infection have no symptoms but do have antibody to CMV (Goodgame, ١٩٩٣).

Circulating lymphocytes, monocytes, and polymorphonuclear leukocytes all probably contain latent virus (Merigan and Resta, ١٩٩٠). Organs at risk for subsequent CMV disease, including the gastrointestinal tract, may contain latent virus that may cause local disease with reactivation (Poland *et.al,* ١٩٩٠).

The pathogenesis of CMV infection must be viewed as a lytic viral infection resulting from a failure of host immunity to control effectively viral replication and spread. However, it is almost a certainty that CMV exerts its pathogenetic potential through non-lytic mechanisms as well. These may include modulation of normal host cell functions by limited expression of its genome, and perhaps

by affecting neighbouring cellular function through the induction of cytokine production. Other pathways of host cell damage may include the generation of host derived immunopathological responses that result in significant organ damage (Grundy and Shanley, ١٩٨٧).

Pathological findings in CMV infection include evidence of viral infection in almost all organ systems (Britt and Alford, ١٩٩٥). Histological findings include large refractile cells (cytomegalic) with so-called owl's eye intranuclear inclusions (Brian and Collier, ١٩٩٨).

٢-٣-٢-٦ Immunity to CMV

Antibodies to CMV occur in most human sera. CMV-specific Abs of the IgM, IgA and IgG classes have all been detected. Reactivation of latent infection occurs in the presence of humoral immunity or Cell-mediated immunity is depressed with primary CMV infections. It may take several months for cellular response to recover. The present of Abs in breast milk dose not prevent transmission of infection to breast-feeding infants. Maternal Abs protects more against development of serious disease in the infant than viral transmission (Brooks *et. al*., ٢٠٠٤).

٢-٤ Laboratory Diagnosis

The diagnosis of CMV relies either on demonstration of the agent (virus, viral proteins, and nucleic acids) in body fluids and /or tissue or on serological responses in a patient with clinical findings consistent with CMV infections. Because of the ubiquitous nature of CMV, it is important that a distinction be made between the detection of CMV and demonstration of CMV in the context of an infectious syndrome compatible with CMV. Multiple methods of recovering virus have been employed (Pass and Britt, ١٩٩٥).

I-Virus isolation; - Urine, saliva, blood and biopsy samples can be used in isolation. Urine should be collected in a sterile container without additives (Demmler *et. al.,* ١٩٩٨). Saliva samples should first be soaked on to swab, which is then broken off into transport medium (Alder and Wang, ١٩٩٦). Blood should be collected into a heparinized bottles (some phenolic preservatives found in proprietary pathology blood bottles may be toxic to blood cultures) containing ٥٠٠ units of heparin (Einhorn, ١٩٨٤). Tissue biopsies should be placed in sterile plastic containers (Hashiro *et. al.,* ١٩٧٩). The specimens have been treated as follows:-

(a)Cell culture- HCMV has been cultured in human cells only, and previous claims that CMV could be grown in other animal cells have not been substantiated (Dunkel *et. al.,* ١٩٩٨). Although CMV can be readily cultured in human fibroblast cells, growth is characteristically slow. It may require ١ to ٤ weeks of growth to develop the typical cytopathic changes in tissue culture. CMV produces characteristic infected cells, which are large, rounded, and contain “ground glass”- appearing inclusions in the nucleus. These

infected cells are the hallmark of CMV and indicate the presence of CMV in the sample (Patel *et. al.*, ١٩٩٦).

CMV can be readily isolated from urine, mouth swabs, buffy coat, cervical tissue, and tissues obtained by biopsy or at post mortem examination. Virus is demonstrable even in the presence of neutralizing antibody (Mandell *et. al.*, ٢٠٠٠).

The growth of CMV from throat, urine, or blood is an abnormal finding. But only culture from blood is highly suggestive of pathogenic CMV infection because CMV in throat or urine is frequently associated with asymptomatic infection. Patient recovering from acute CMV mononucleosis may shed CMV in the urine and throat for several weeks (Patel *et. al.*, ١٩٩٦).

(B) DEAFF (Detection of early antigen fluorescent foci):- This is a method used for the early diagnosis of CMV infection (Kettering *et. al.,* ١٩٧٧). In immunocompromised patients, a sensitivity of ٧٨٪ and a specificity of ١٠٠٪ have been claimed (Rao, *et. al.,* ١٩٧٧). The specimen is inoculated into cell culture, which is examined ٢٤ hours later by immunofluorescence for expressed CMV encoded early proteins. The monoclonal antibodies must be able to cover most, if not all strains of CMV. Rapid culture methods other than the DEAFF tests are also available (Murray *et. al.,* ٢٠٠٣).

(C) Histopathology:- Histologic examination of Wright-Giemsa, hematoxylin-eosin, or Papanicolaou stained lung or other biopsy specimens can be useful in the diagnosis of localized CMV organ disease (Deibel *et. al.,* ١٩٧٤). Characteristic large cells (cytomegalic

cells) with basophilic intranuclear inclusions and, on occasion, eosinophilic cytoplasmic inclusions can be seen in routine sections of biopsy or outopsy material (Myerson and Hackman, ١٩٨٤). The nuclear inclusion has the appearance of an “owl’s eye” because it has marginated chromatin that is typically surrounded by a clear halo that extends to the nuclear membrane. The procedure is rapid and simple to perform, and the presence of characteristic cytologic changes suggests CMV infection and correlates with active disease in most cases, although additional virologic or serologic confirmation is suggested (Landini, ١٩٩٣). Overall, histopathologic diagnosis of CMV disease is relatively insensitive, and since CMV can infect tissues without producing morphologic changes, failure to find typical cytomegalic cells does not exclude the possibility of CMV infection (Murray *et. al.,* ٢٠٠٣).

(d) Tissue immunofluorescence: - Infected lung and liver cells may be stained by specific anti-CMV antibodies. Broncheolavage specimens can also be examined in this manner. Results of high sensitivity and specificity are possible (Booth *et. al.,* ١٩٨٢).

(e) Electron microscopy: - Virions in the urine of congenitally infected infants may be visualized by EM in up to ٨٠٪ of cases. However this is of no real value as rapid diagnosis is not required. In immunocompromised individuals though, the viral titters are

generally lower than neonates and other herpesviruses are often present in the urine (Ho, ١٩٩١).

(f) ELISAs for detection CMV antigens in the urine: - these tests carry low sensitivity, as CMV is complexed to ß٢-microglobulin in the urine (Stagno *et. al.*, ١٩٨٥).

(g) Molecular amplification: PCR is currently the most widely used molecular method for the detection of CMV DNA and mRNAs. Many in-house and some commercial qualitative assays have been developed, and the sensitivity and specificity of PCR for diagnosis of active CMV infection have been evaluated (Long *el. al.,* ١٩٩٨; Solano *et. al.*, ٢٠٠١). Amplification has been performed with a variety of primer pairs from the immediate-early antigen ١, the major immediate-early antigen, glycoproteins B and H, the EcoRI D fragment, the HindIII X fragment, phosphoprotein ٦٥ (pp٦٥) , pp٦٧, and the major capsid protein gene fragments(Wolf and Spector, ١٩٩٢). (Blok *et. al.,* ٢٠٠٠) revealed that the sensitivity of the assay was increased by amplifying genomic regions from both the immediate-early and the late CMV genes or by using nested primers to a single gene fragment. The use of both gene fragments enabled the detection of a variety of clinical isolates, indicating that strain variability is not a limiting factor for PCR diagnosis of CMV (Murray *et. al.,* ٢٠٠٣) .

(h) CMV antigenaemia test: - Techniques of viral antigen detection have been relatively limited with the exception of the antigenaemia assay. This test is based upon the detection of pp٦٥, a structural protein expressed on the surface of infected polymorphonyclear lymphocytes (Gerna and Zipeto, ١٩٩١). The number of infected leukocytes present had been reported to correlate with the severity of infection. The main advantage of this test is that it is very rapid so that a result can be available within the same day. As a result, this test is now widely used especially in the monitoring of transplant recipients (Miller and Rossier, ١٩٩١).

II- Endoscopic Examination: The definitive diagnosis of CMV gastrointestinal disease depends on invasive procedures and biopsies. Upper gastrointestinal endoscopic examinations (Theise *et. al.*, ١٩٩١; Bonacini *et. al.*, ١٩٩١; Goodgame *et. al.*, ١٩٩١) and lower gastrointestinal endoscopic examinations (Lepinski and Hamilton, ١٩٩٠; Connolly *et. al.*, ١٩٩٠) have frequently been used to evaluate gastrointestinal symptoms and signs in patients at risk for CMV infection. The mucosa may appear grossly normal despite the presence of cytomegalic cells seen in biopsy specimens (Dieterich and Rahmin, ١٩٩١). However, CMV disease usually appears as a mucosal erosion or ulceration. Because no endoscopic feature is pathognomonic for CMV disease, the diagnosis depends on the change detection in endoscopic biopsy specimens. Endoscopic examination is generally well tolerated, but perforation of a CMV esophageal ulcer after upper

gastrointestinal endoscopic examination has been reported (Parente *et. al*., ١٩٩١).

III- Serology; - CMV IgM antibodies are detected in primary infection and last for ٣ - ٤ months. It is not detectable in recurrent infection except in immunocompromised patients where it is detectable in about a third of the cases (Hodinka, ١٩٩٩). CMV IgM may be undetectable in primary infection in immunocompromised individuals. Solid phase sandwich or antibody capture ELISA is now in routine use (Dylewski *et. al.*, ١٩٨٤). Interference by rheumatoid factor should be excluded. CMV IgM can be sought for in the cord blood samples from infants who are suspected of being congenitally infected and the titer present is generally related to the outcome (Grangeot-Keros *et. al.,* ١٩٩٧).

CMV IgG is produced early in primary infection and persists lifelong (Rasmussen *et. al*., ١٩٨٢). The detection of CMV IgG is useful as an "immune status screen" (seropositive individuals are not protected from reactivation of reinfection). Rising titers of IgM can be used as markers of acute infection (Landini and Rossier, ١٩٨٨). This is particularly useful in diagnosing recurrent infections in normal individuals, and in immunocompromised patients who may not develop an IgM response to primary infection (Meyers, ١٩٩١). Various methods are used for detecting CMV IgG including CFT, IFT, latex agglutination, and ELISAs (Murray *et. al*., ٢٠٠٣).

Chapter Three

Materials & Methods

Chapter Three

Materials & Methods

٣-١ Study Design and Study Groups

٣-١-١ Patients

A hundred patients with peptic ulcer disease selected randomly to study the role of CMV in peptic ulcer. They were ٤٢, ١٨ and ٤٠ patients from Merjan Hospital, Babylon Teaching Hospital and Al-Karama Teaching Hospital respectively. The diagnoses of peptic ulcers were made by clinical history and upper GIT endoscopy. They were ٧٦ males and ٢٤ females. The ages of patients were between (١٦- ٧٠) years old. The mean age was ٣٨ years old. Upper GIT endoscopy was done for all patients in this study. We detected ٢٣ and ٧٧ gastric ulcer and duodenal ulcer patients respectively. A blood

sample was taken from each patient, while gastric biopsies were taken from ٣٨ patients only.

The study was conducted from November ٢٠٠٤ to July ٢٠٠٥ in the Babylon Teaching Hospital, Merjan Hospital in Hilla City and Al-Karama Teaching Hospital in Baghdad.

The serum sample was separated from blood samples and stored at -١٠ºC in the laboratory of Merjan Hospital and Babylon Teaching Hospital. The ELISA test was done in the Public Health Laboratory in Hilla. Each biopsy specimen was divided into two parts. One was used for histological examination (hematoxylin – eosin stain), which was done in the Babylon Teaching Hospital and Al-Karama Teaching Hospital. The other part of biopsy was examined by fluorescent technique, in veterinary laboratory in Baghdad.

٣-١-٢ Healthy control group

Fifty healthy people were chosen in this study. They had no symptoms of peptic ulcer diseases. They were almost similar to patients in ages, sex, social status, economical status and their residence

The following information was taken from each person in this study: name, age, sex, residence, smoking, alcohol, immune suppression drugs, any other disease and type of sample (blood, biopsy). The collected information for peptic ulcer patients is presented in Table (٣-١)

Table (٣-١) Collected Information for Peptic Ulcer Patients

Sex	Residence		Smoking	Drinking alcohol	Immune suppression drugs	Type of ulcer	
	Rural	Urban				Stomach	Duodenum
Male	٢٨	٤٨	١٨	٠	٠	١٨	٥٨
female	٨	١٦	٣	٠	٠	٥	١٩
Total	٣٦	٦٤	٢١	٠	٠	٢٣	٧٧

٣-٢ Materials

٣-٢-١ Equipments and tools

The following equipments and tools were used in this study:

Device	company	Origin
Automatic Pipette ٥, ٥٠, ١٠٠, ٥٠٠ µl	Slamed	Germany
Centrifuge	Heraeus	Germany
ELISA reader	Beckman coulter	Austria
Endoscopy	Olympus	Japan
Fluorescent microscope	Olympus	Japan
Freezer	Liebher	Austria
Gastric tube	Olympus	Japan
Gastric-biopsies(forceps)	Olympus	Japan
Incubator	Heareus	Austria
Microscope	Olympus	Japan
Microtiter plate washer	Beckman coulter	Austria
Refrigerator	Liebher	Austria

٣-٢-٢ Cytomegalovirus (CMV) IgG and IgM ELISA kits:

The ELISA Cytomegalovirus (IgG, IgM) kits were provided by BC (BioCheck) ٣٢٣ Vintage Park Dr. Foster City, CA ٩٤٤٠٤ (USA).

٣-٢-٢-١ Contents of the test kit:

A) Cytomegalovirus IgG ELISA kit:

- **Microtiter Strips**: Strips with ٨ breakable wells each, coated with purified CMV antigen.
- **Negative Calibrator**: ٠ IU/ml (١٥٠µl).
- **Positive Calibrator:** ٦ IU/ml (١٥٠µl).
- **Positive Calibrator:** ١٨ IU/ml (١٥٠µl).
- **Cut-off Calibrator:**١.٢IU/ml(١٥٠µl).CMV/IgG index= ١.٠
- **Negative control:** (١٥٠µl).

- **Positive control:** (١٥٠µl).
- **Enzyme conjugate:** ١٢ ml, anti-human-IgG-HRP (rabbit), conjugated to protein-containing buffer solution.
- **Tetramethylbenzidin (TMB) substrate solution:** ١١ ml.
- **TMB stop solution:** ١١ ml, ١N HCl.
- **Sample Diluent:** ٢٢ ml, PBS buffer.
- **Washing Buffer Concentrates (٢٠x):** ٥٠ ml, PBS+Tween ٢٠, ٢٠x concentrate.
- **Plastic Foils:** ٢ pieces to cover the microtiter strips during the incubation.
- **Plastic Bag:** Resealable, for the dry storage of non-used strips.

B) Cytomegalovirus IgM ELISA kits:

- **Microtiter Strips**: Strips with ٨ breakable wells each, coated with purified CMV antigen.
- **Cut-off Calibrator:**١.٢IU/ml(١٥٠µl).CMV/IgMindex = ١.٠ .
- **Negative control:** (١٥٠µl).
- **Positive control:** (١٥٠µl).
- **Enzyme conjugate:** ١٢ ml, anti-human-IgM-HRP (rabbit), conjugated to protein-containing buffer solution.
- **Tetramethylbenzidin (TMB) substrate solution:** ١١ ml.

- **TMB stop solution:** ١١ ml, ١N HCl.
- **Sample Diluent:** ٢٢ ml, PBS buffer.
- **Washing Buffer Concentrates (٢٠x):** ٥٠ ml, PBS+Tween ٢٠, ٢٠x concentrate.
- **Plastic Foils:** ٢ pieces to cover the microtiter strips during the incubation.
- **Plastic Bag:** Resealable, for the dry storage of non-used strips .

٣-٢-٣ Fluorescent stains

The fluorescent stains of CMV (IgG, IgM) were provided by Euroimmun- Medizinische Labordiagnostika A G. Des Plaines, IL٦٠٠١٦, USA.

٣-٢-٣-١ Contain of fluorescent stains: -

١. Conjugate (٥X) IgG ١:٥ in PBS/ tween

٢. Conjugate (٥X) IgM ١:٥ in PBS/ tween

٣. Phosphate Buffered saline (PBS) pH ٧.٤ ±٠.٢, ٠.٠١ M

٣-٣ Methods

٣-٣-١ Blood samples collection and storage

Five ml blood samples were obtained by veinpunture from all studied people after cleaning the skin with ٧٠٪ alcohol, then the blood samples were stored in plastic tubes. Blood samples were allowed to clot undisturbed for about ١ h at room temperature. Then the clot was loosed gently from the tube wall by means of a wooden stick, then was centrifuged for ١٠ min at ١٢٠٠ r.p.m.. Then the serum was transferred into other tubes (Voler and Bidwell, ١٩٨٥; Cremer, ١٩٨٥; Starr and Friedman, ١٩٨٥).

After the serum samples were collected, they were stored at – ١٠°C until they were tested by ELISA for quantitative determination of anti Cytomegalovirus IgG and IgM, and fluorescent technique.

٣-٣-٢ Obtaining of biopsies

The endoscopy of the upper part of GIT was conducted in the morning. The patient did not eat food for the whole night and morning. The patient lied on his back and was given pharyngeal anesthetic that contained of ٠.٢٪ Lidocaine (Xylocain) for easy insertion of endoscopy. The patient was turned on his left side putting his left hand under his head. He opened his mouth and circular hollow plastic mouth piece was put between the upper and lower teeth. The gastric tubes was inserted into mouth, throat, esophagus, stomach and duodenum to find ulcers then the gastric tube was pulled up to stay in antrum. A transverse slot closed with valve was found in gastric tube. After opening the valve, a thin cable was inserted through the transverse slot. The end of the cable which entered stomach contained tongs its hatch ٧ mm and it was controlled by the other end. The tongs extended to the mucosa layer to the gastric antrum to take a biopsy from stomach ulcer patient. However, the tongs extended to the mucosa layer of the duodenum to take a biopsy from the ulcer of duodenal ulcer patients. The collected biopsy was divided into two parts. One of the two parts was put in a plane tube filled with formalin, the other part was kept in saline soaked cotton gauze (Qais *et.al.,*١٩٩٠).

٣-٣-٣ ELISA procedure

BioCheck instructions in ELISA of IgG (Catalog Number: BC-١٠٨٩), and IgM (Catalog Number: BC-١٠٩١) were followed:

٣-٣-٣-١ Preparation of samples and reagents

١) Samples

Before use, the patient's serum samples were diluted ١:٤٠ with buffer serum diluent (the best chequre-board titration) (٥µl of sample + ٢٠٠µl buffer).

٢)Wash buffer

The wash buffer was diluted with distilled water ١ to ٢٠ (١٠ ml concentrate + ١٩٠ ml distilled water).

٣-٣-٣-٢ Assay procedure

١. A sufficient amount of microtiter wells was prepared for the standard controls and samples.
٢. ١:٤٠ dilution of test samples, Negative Control, Positive Control, and Calibrator by adding ٥µl of the sample to ٢٠٠µl of sample diluent were prepared.
٣. ١٠٠ µl of diluted sera, Calibrator, and Controls were dispensed into the appropriate wells of the strip.
٤. The plates were covered with the enclosed foil and incubated at ٣٧ºC for ٣٠ minutes.

٥. At the end of incubation period, liquid was removed from all wells. were rinsed and flicked the microtiter wells ٤ times with diluted wash buffer (١x) and then once with distilled water.

٦. ١٠٠µl of Enzyme Conjugate was added to each well in sequence.

٧. The plates were covered with enclosed foil and incubated for ٣٠ minutes at ٣٧ºC.

٨. We rinsed and flicked the microtiter wells ٤ times with diluted wash buffer (١x) and then one time with distilled water.

٩. ١٠٠ µl of TMB reagent was added into each well.

١٠. The plates were covered with enclosed foil and incubated for ١٥ minute at ٣٧ºC.

١١. The reaction was stopped by adding ١٠٠ µl of TMB stop solution to each well.

١٢. The microtitter strips were shaken gently and the reading was taken at ٤٥٠ nm within ١٥ minutes.

٣-٣-٣-٣ Calculation of results

١- Cut-off calibrator value x_c was calculated.

٢- Positive control (x_p), negative control (x_n) and patient samples (x_s) were calculated.

٣- The CMV IgG and IgM Index of each determination by dividing the values of each sample (x) by calibrator value (x_c) were calculate**d.**

٣-٣-٣-٤ Quantitative determination of CMV IgG

For a quantitative determination of anti-CMV IgG levels of positive specimens in IU/ml, the O.D. of cut-off, negative, and positive calibrators are plotted on the Y-axis of a graph against their corresponding anti-CMV IgG concentrations of ٠, ١.٢, ٦, and ١٨ IU/ml on the X-axis. The estimates of levels in patient sera are read off the graph using their individual O.D. values.

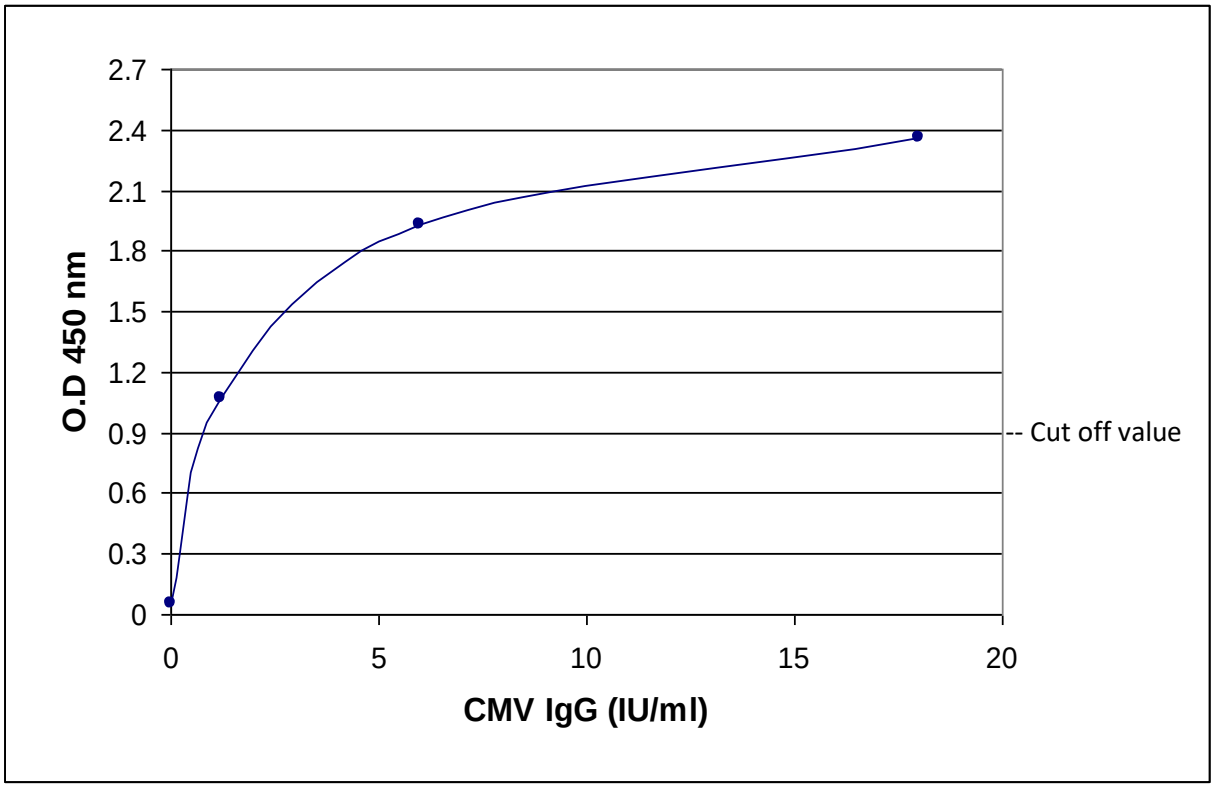


Figure ٣-١ Standard Curve of Anti- CMV IgG (positive ≥ ١ And negative < ٠.٩)

٣-٣-٣-٥ Interpretation of the results

The results of each sample were assessed as the following (Voler and Bidwell, ١٩٨٥; Cremer, ١٩٨٥; Starr and Friedman, ١٩٨٥).

١. Negative: CMV IgG and IgM Index less than ٠.٩٠ is seronegative for IgG and IgM antibody to CMV. (<١.٢ IU/ml).

٢. Equivocal: CMV IgG and IgM Index between ٠.٩١-٠.٩٩ is equivocal. Sample should be retested.

٣. Positive: CMV IgG and IgM Index of ١.٠٠ or greater,or IU value greater than ١.٢ is seropositive. It indicates prior exposure to the CMV. (>١.٢ IU/ml).

٣-٣-٤ Fluorescent assay procedure

It was carried out depending on Euroimmun- Medizinische Labordiagnostika AG. Des Plaines, IL٦٠٠١٦, USA.

Assay procedure ٣-٣-٤-١

١. Cryostat chuck was precooled in liquid nitrogen for about ١ min.
٢. The biopsy specimen was immobilized on top of the precooled cryostat chuck by using a few drop of water, as the water began to freeze, the chuck was inverted to eliminate excess water.

٣. The chuck was placed in liquid nitrogen to snap-freeze the tissue. As a rule, the tissue can be considered adequately frozen when the liquid nitrogen stops bubbling. At this stage, the frozen specimen can be stored at –٧٠ °C for as long as necessary.

٤. ٦-µm sections of frozen biopsy specimen were cut on a tissue cryostat at - ٢٠°C

٥. Six sections were placed on each standard histologic slide. The sections were separated from each other by liquid embroidery fluid lines.

٦. The slides were allowed to air dry for at least ١٠ min.

٧. Overlay each tissue section with appropriate fluorescent antiserum.

٨. The slides were incubated for ٢٠ min. In a closed humidity chamber at room temperature.

٩. The unreacted antiserum off the sections was washed by dipping the slides sequentially into three jars of ٠.٠١ M phosphate-buffered saline (PBS), pH ٧.٢. After the three rinses, the slides are immersed in a fourth jar of PBS for ١٥ min.

١٠. The slides were drained and wiped excess buffer from around the sections with cotton gauze. Cover slips was put on the slides, with drop of buffered glycerol (٩٠٪ glycerol) as the mounting medium.

١١. The slides were examined with fluorescence microscope. The ideal combination of excitation and barrier filters should be determined

by the absorption and emission spectra of the fluorochrome (Edwin *et. al.,* ١٩٨٥; Noel *et. al.,* ١٩٨٦) .

٣-٣-٥ Procedure of histopathlogical changes assessment

The slides of biopsy were prepared according to the procedure given by Qais *et.al.* (١٩٩٠).

٣-٣-٥-١ Schedule for automatic tissue processor: -

١. (٢ hrs) formalin
٢. (١ hr) ٧٠٪ alcohol.
٣. (١ hr) ٩٠٪ alcohol.
٤. (٢ hrs) absolute alcohol First change
٥. (٢ hrs) absolute alcohol Second change
٦. (٢ hrs) absolute alcohol Third change
٧. (١ hr)Xylol First change
٨. (٢ hrs)Xylol Second change
٩. (٢ hrs)Xylol Third change
١٠. (٢ hrs) Paraffin wax First change
١١. (٢ hrs) Paraffin wax Second change
١٢. (٢ hrs) Paraffin wax Third change

٣-٣-٥-٢ Scheme for rapid method (Urgent tissue): -

Thin slices of tissue (٣-٥mm thick) were used.

١. It was heated in formalin saline (just of the boil) ١٠-١٥ min.
٢. It was dehydrated in acetone at ٥٦°C in wax oven, several changes in ٢ hours.
٣. It was cleared in Xylol at ٥٦ °C (٢ changes) ٢٠-٢٥ minutes.
٤. It was impregnated with wax (several changes) ١ ½-٢ hours.

Preferably vacuum embed for ١-٢ hours, blocked out and cut.

٣-٣-٥-٣ Haematoxlin and eosin staining method:-

١. Sections were dewaxed in Xylol, then treaed with graded alcohol and placed in water.
٢. It was stained in haematoxlin for ٣-١٠ minutes.
٣. It was washed well in running tap water.
٤. It was removed excess stain by differentiating in ١٪ acid alcohol (١٪ HCl in ٧٠٪ alcohol) for ٥ to ١٠ minutes.
٥. It was washed well in tap water until sections regained blue color, ٥ minutes.
٦. It was stained in ١٪ eosin for ٢-٥ minutes.
٧. It was dehydrated slowly through gradede alcohol to Xylol.
٨. It was mounted in Canada Balasm.

Two pathologists examined the prepared slides, in order to read the tissue changes according to Sydney classification.

٣-٣-٥-٤ Preparation of eosin stain

١٠ gms eosin was dissolved in ١٠٠٠ ml. (١ liter**)** Distilled water.

٣-٣-٥ Statistical analysis

Differences between means were compared by t and $X^{٢}$ tests under confidence level of ٠.٩٥; the P value ≤ ٠.٠٥ was considered a significant difference and P value > ٠.٠٥ was considered a non-significant difference.

Chapter Four

Results

Chapter Four

Results

٤-١ Clinical Findings

The diagnosis of peptic ulcer were made by proper clinical history which had been done by a physician. In addition the diagnosis of peptic ulcer were made by endoscope finding. We detected ١٠٠ chronic peptic ulcer of whom ٧٧ duodenal ulcer and ٢٣ gastric ulcer.

٤-٢ Anti CMV Antibody Titers in Peptic Ulcer Patients According to Age Groups

The anti CMV antibody (IgG and IgM) in ١٠٠ peptic ulcer patients and ٥٠ normal people presented in this study is shown in Figure (٤-١), (٤-٢) and (٤-٣). The age of patients ranged between (١٦-٧٠) years. The mean age of patients was ٣٨ years old. Anti CMV IgG antibody was positive in all patients. There were ٣,٢١, ٤٢, ٢١, ١١and ٢ patients in the (١٠-٢٠), (٢١-٣٠), (٣١-٤٠), (٤١-٥٠), (٥١-٦٠) and (٦١-٧٠) years age groups respectively. Anti CMV IgG in healthy control people was negative except in ٦ people. There were only ٢, ٣and ١ people with positive results in (١٠-٢٠), (٢١-٣٠) and (٣١-٤٠) years age groups respectively.

Anti CMV IgM was positive in ١١ patients only. There were ١,٤,٢ and ٤ in (١٠-٢٠), (٢١-٣٠), (٣١-٤٠), and (٤١-٥٠) years age groups respectively, whereas all normal people had negative IgM.

Table (٤-١) shows the differences in mean values of IgG and IgM of CMV between peptic ulcer patients and healthy control people of different age groups which were significant at ٠.٠١ level.

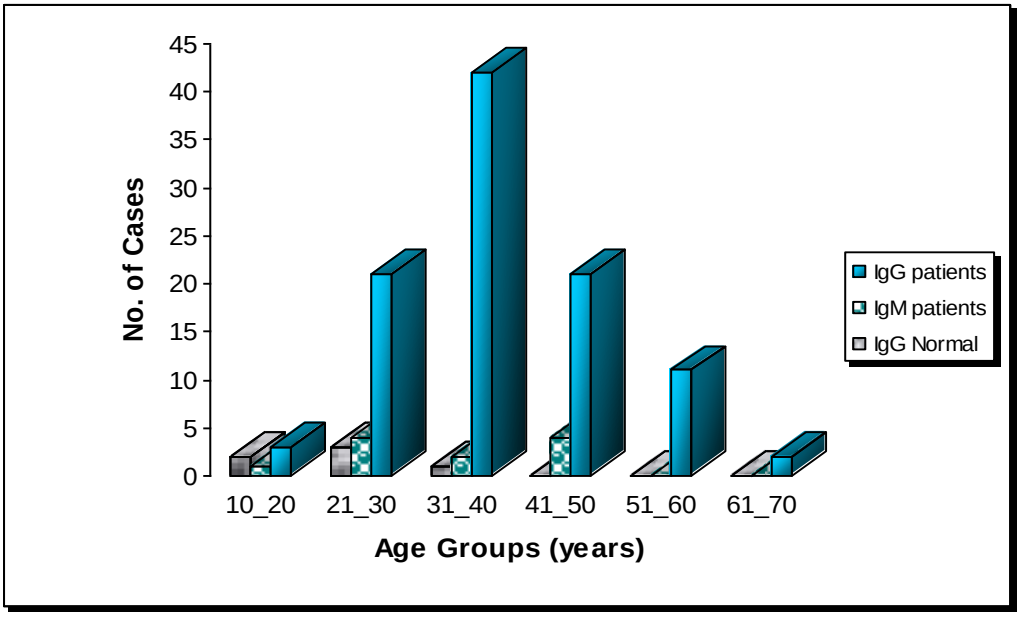
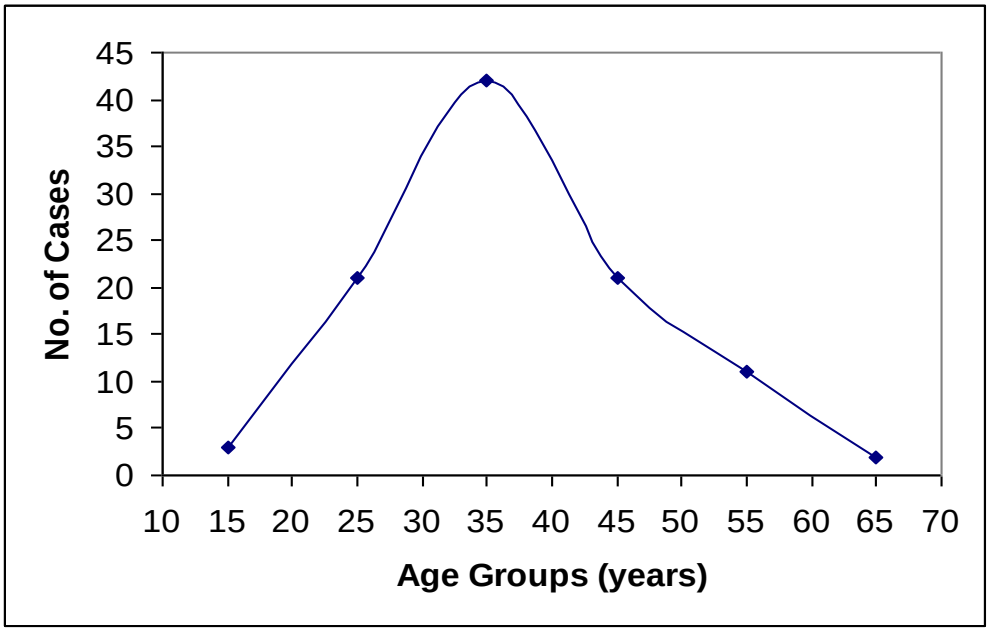
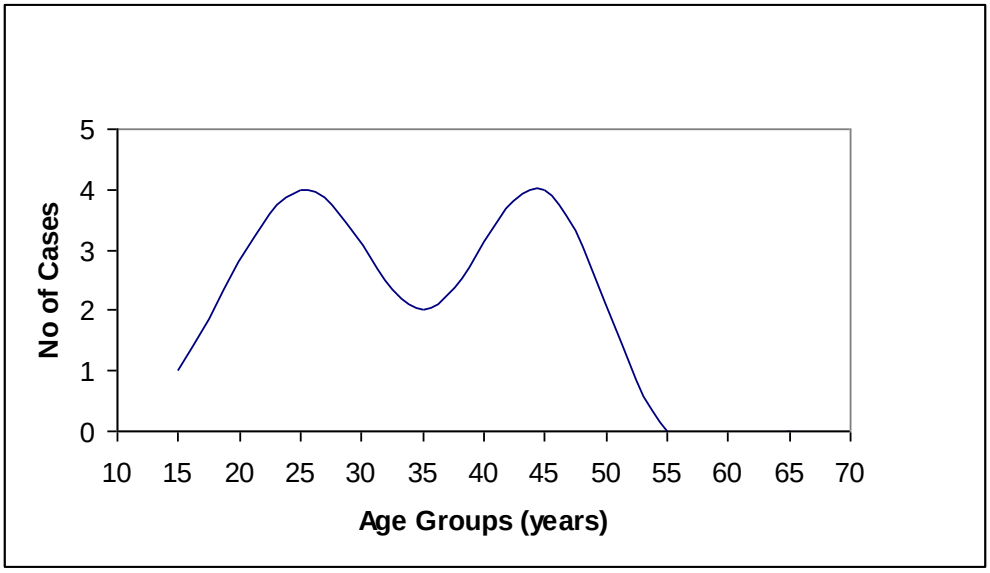


Figure (٤-١) Anti CMV Antibody (IgG and IgM) Titers for Peptic Ulcer Patients and Healthy People According to Age Groups



Figure(٤-٢) Age Distribution of Peptic Ulcer Patients with Positive Anti CMV Antibody (IgG).



Figure(٤-٣) Age Distribution of Peptic Ulcer Patients with Positive Anti CMV Antibody (IgM).

Table (٤-١) Means and Standard Deviation of Antibodies Titer Values of Anti CMV IgG and IgM Antibody in Peptic Ulcer Patients and Healthy Control People

Treat	Number	Mean of Titer	± Std. Deviation	± Std. Error Mean	P value
IgG P	١٠٠	١.٩٧٨٠	٠.٣٢١٦	٣.٢١٦E-٠٢	< ٠.٠١**
C	٥٠	٠.٧٣٥١	٠.٣٠٨٧	٤.٣٦٦E-٠٢	

IgM	P	١٠٠	٠.٧٠٨٩	٠.٢٩٣٤	٢.٩٣٤E-٠٢	< ٠.٠١**
	C	٥٠	٠.٥١٦٦	٠.٢٠٧٦	٢.٩٣٦E-٠٢	

** Highly significant

P: peptic ulcer patient

C: healthy control people

٤-٣ Anti CMV Antibody Titers in Peptic Ulcer Patients According to Sexes

The sex distribution of peptic ulcer patients is shown in Figure (٤-٤). There were ٧٦ males and ٢٤ females. The male to female ratio was ٣:١.

The IgG of CMV was positive in all male and female patients, while IgM of CMV was positive only in ٨ male and ٣ female patients Figure (٤-٥). Table (٤-٢) shows that there was a significant difference between male and female immune response P< ٠.٠١ level.

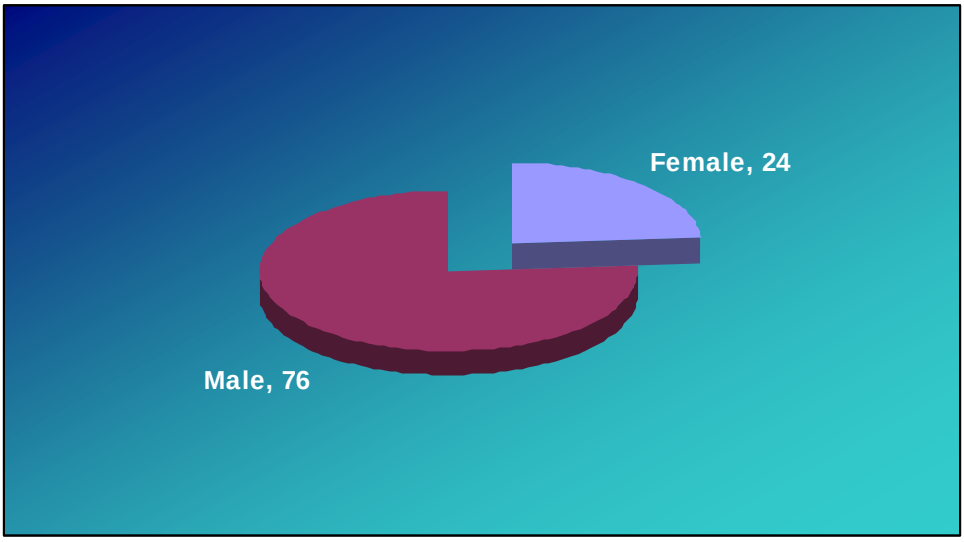


Figure (٤-٤) Sex Distribution of Peptic Ulcer Patients with Positive Anti CMV Antibody (IgG).

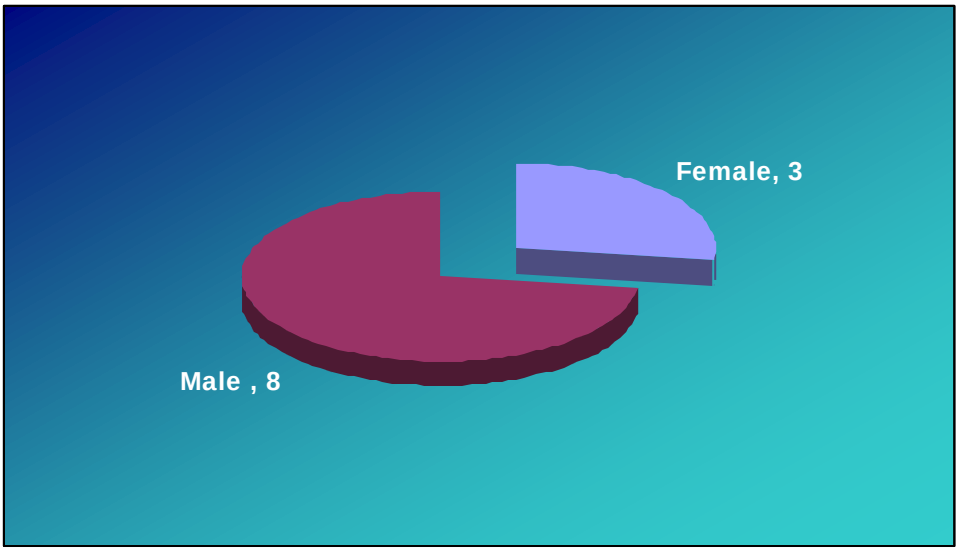


Figure (٤-٥) Sex Distribution of Peptic Ulcer Patients with Positive Anti CMV Antibody (IgM).

Table (٤-٢) Comparison Between Number of Male and Female Peptic Ulcer Patients Infected by CMV.

Sex of patients	Number of peptic ulcer patients infected by CMV	P value between male and female
Male	٧٦	< ٠.٠١**
female	٢٤	

** Highly significant

٤-٤ Residential Distribution of Peptic Ulcer Patients

The residential distribution of peptic ulcer patients is shown in Figure (٤-٦). The rural resident's patients were ٣٦ while urban resident's patients were ٦٤.

Table (٤-٣) shows the number of peptic ulcer patients who have positive IgG and IgM of CMV according to their residents. The number of rural and urban patients for IgG and IgM were ٣٦, ٦٤ and ٤, ٧ respectively.

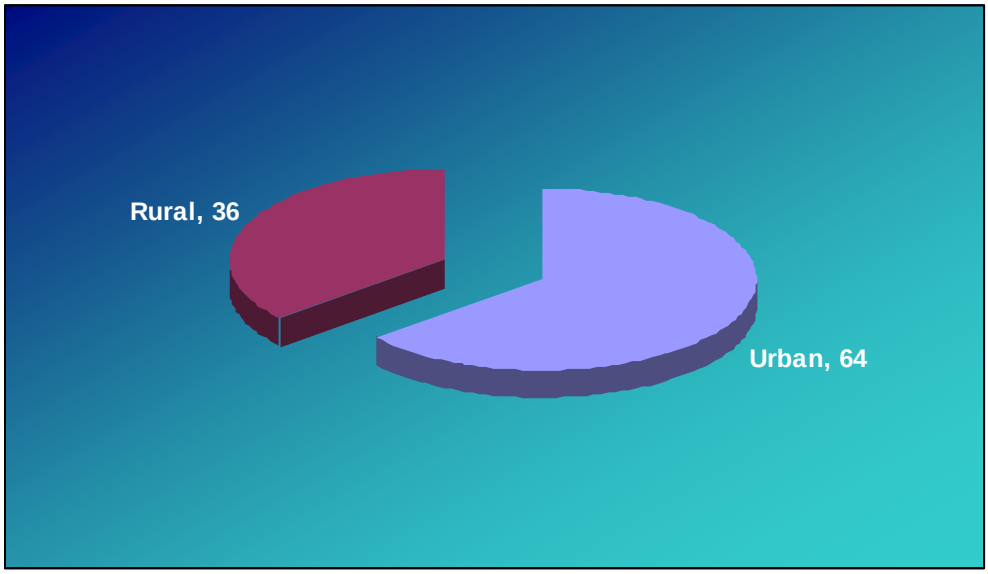


Figure (٤-٦) Residential Distribution of Peptic Ulcer Patients

Table (٤-٣) The Residential Distribution of Peptic Ulcer Infected with CMV.

Residence	IgG	IgM
Rural	٣٦	٤
Urban	٦٤	٧

P value	< ٠.٠١**	> ٠.٠٥ N.S

** Highly significant N.S Non significant

٤-٥ Histopathological changes

Figure (٤-٧) shows that the normal state of inflammatory cells spread in mucosa layer in stomach antrum. Inflammatory cells spread very little through the lamina propria. Figure (٤-٨) and Figure (٤-٩) show the giant cell formation in gastric antrum and duodenum of gastric and duodenal ulcer patient respectively infected with CMV.

٤-٦ Fluorescent Technique

Figure (٤-١٠) and Figure (٤-١١) shows section stained with specific anti CMV IgG and IgM respectively show the presence of virus specific antigen in the stomach tissue by fluorescent microscope

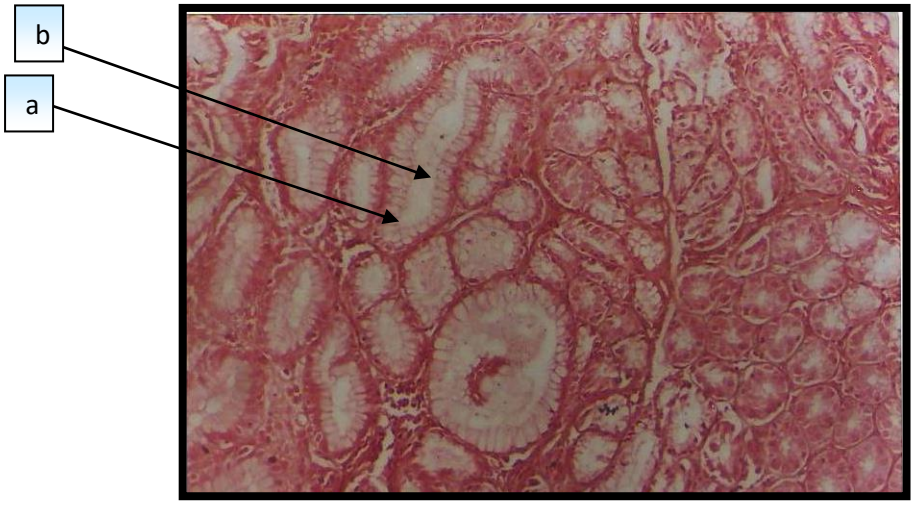


Figure (٤-٧) A Section of Mucosa Layer in Stomach Antrum of Normal Person (Hematoxylin –Eosin ٤٠٠ X)

a: Inflammatory cells b: Lamina propria

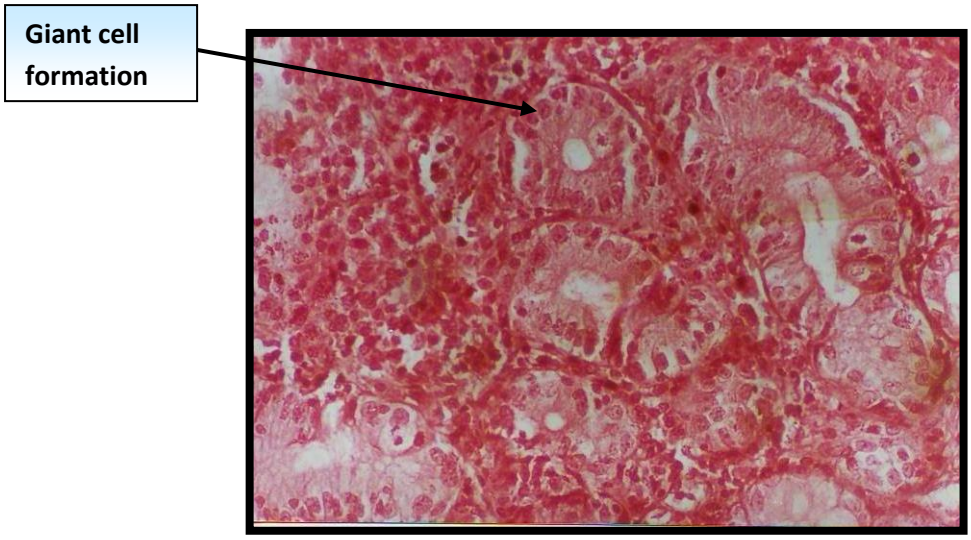


Figure (٤-٨) A Section of Mucosa Layer in Stomach Antrum of Stomach Ulcer Patient Infected with CMV (Hematoxylin –Eosin ٤٠٠ X).

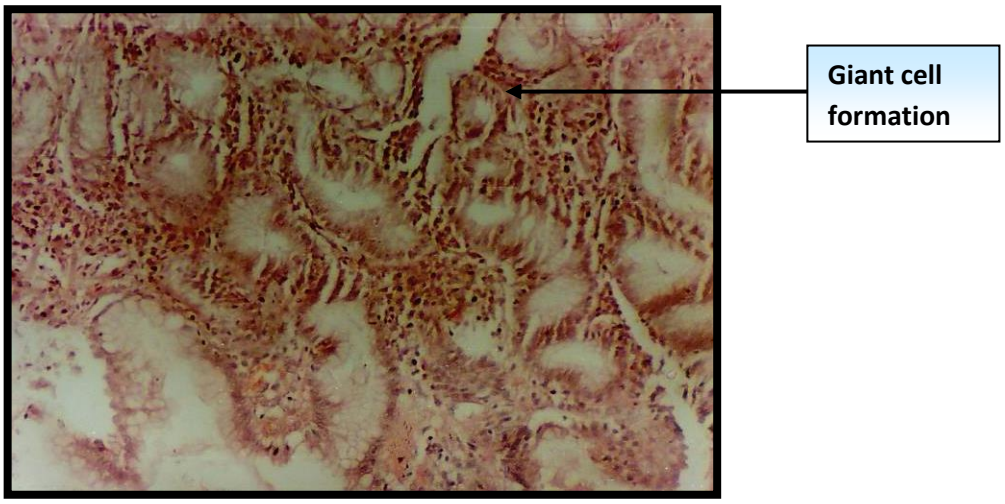
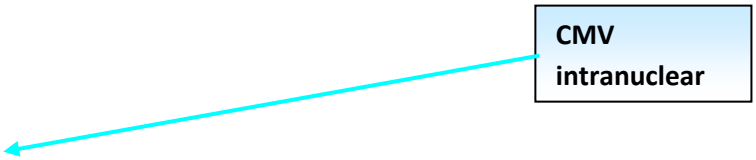


Figure (٤-٩) A Section of Mucosa Layer in Duodenum of Duodenal Ulcer Patient Infected with CMV (Hematoxylin –Eosin ٤٠٠ X).



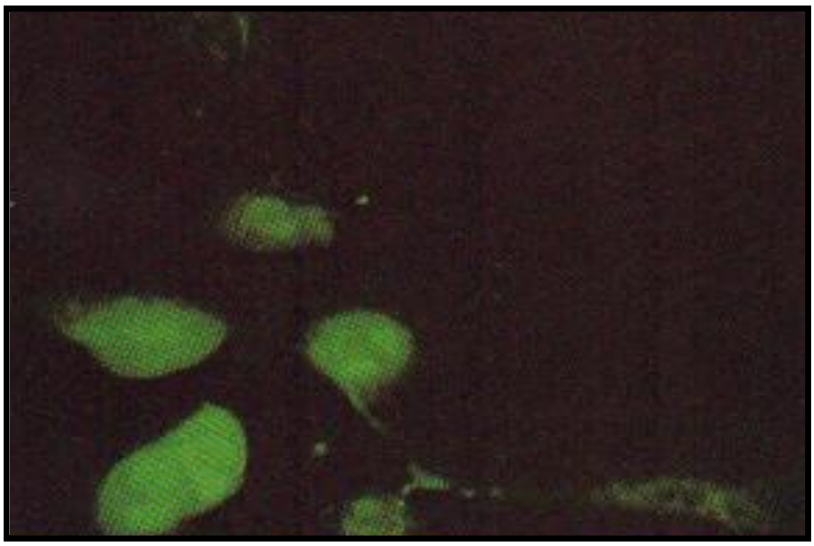


Figure (٤-١٠) Stomach Section Stained with Specific Anti CMV IgG .

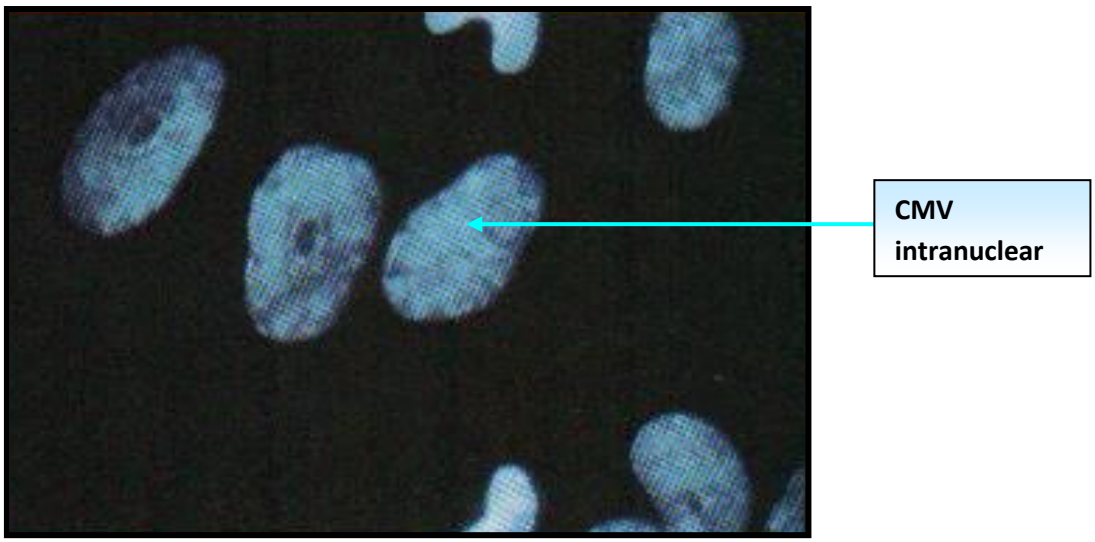


Figure (٤-١١) Stomach Section Stained with Specific Anti CMV IgM .

Chapter Five

Discussion

Chapter Five

Discussion

٥-١ Anti CMV antibody Titers in Peptic Ulcer Patients According to Age Groups

All research work from different parts of the world had proven that almost all people have had CMV infection during their life from early childhood and become persistent (Hinnant *et. al.,* ١٩٨٦; Cheung and Ng IO, ١٩٩٣; Vancikova and Dvorak, ٢٠٠١). Moreover; other researchers had mentioned that the virus affect mainly the digestive system and believed that the lesion possibly affect alimentary canal and persist dormant in the latent form for life. This latent stage could be activated and results in lesions (vesicles) formation due to different inducing factors which may include stress, physiological, psychological or other disease conditions which results in inhibition of immune status (immune defense mechanisms). Possible relation with other factors that possibly reactivate CMV proliferation from the latent state to form lesion in the gastric tract might be the corner stone of peptic ulcer occurrence. This agrees with Hansen *et. al.,* ١٩٩٤ who had described the reactivation of CMV after some time of dormant stage. In my study a ١٧ years old young patient with high titer of anti CMV IgM antibody, which indicates a primary CMV infection and a lesion was found by endoscopy of the GIT. Eighty four percent of the patients were between ٢٠ and ٥٠ years old. The peak age of incidence was between ٣٠ and ٤٠ years old. This may be due to the fact that stress and complication of life requirements were

more at this age group. The findings of the present work agreed with (Cryer *et. al.,* ١٩٩٢; Laurance *et. al.,* ١٩٩٧; Lin *et. al. ,* ١٩٩٨ ; Dong *et. al.,* ٢٠٠٤) in the fact that the exposure to stress factors in the presence of other materials which cause ulceration such as (chemical materials, effect of diet and drugs) increased as the age increased. This is just part of the important sequence of events that lead to activation of CMV from the dormant (latent) state. Lin *et. al. ,* ١٩٩٨ believed that the effect of *Helicobacter pylori* will increase as the age increase due to colonization of the lesions formed from CMV activation. Arnar *et.al.,* ١٩٩١ had mentioned that its characteristic of reactivate from the latent state thus may have the primary role in the pathogenesis of gastrointestinal lesions in nonimmunocompromised patients. A recent study by Bruno *et. al*.,٢٠٠٥ had suggested that CMV, rather than *Helicobacter pylori*, may be the main causative pathogen of peptic ulcer patients which proved the hypothesis about the role of CMV in peptic or gastric lesions.

Raiha *et.al.,*١٩٩٨ had mentioned that all males and females peptic ulcer patients had positive anti CMV antibodies (IgG) and this was found in our study also. The IgG anti CMV antibody were positive for all peptic ulcer patients (Figure ٤-١) and (Figure ٤-٤). This means that all patients are at chronic state and the presence of the antibody indicate the presence of lesion otherwise the antibody may decline

to a very low level or disappear. The results show that age groups between ٣٠ to ٤٠ years have had anti CMV IgG anti bodies which were greater than those in normal people which indicate that these people have had long experience with stress factors that had led to the proliferation of CMV from the latent state and this was mentioned previously (Goodgame, ١٩٩٣; Gondo, ١٩٩٨; Vancikova and Dvorak, ٢٠٠١; Chiu *et. al.,* ٢٠٠٤) that IgG is an indicator of chronic presence of CMV in the lesion of those people who had suffered from peptic ulcer for long period.

The anti CMV antibody IgM was positive only in ١١ patients in age groups from ١٦-٤٠ years old which represents the acute state of CMV infection which is compatible with the same sequence of CMV infection events as mentioned by (Vancikova and Dvorak, ٢٠٠١; Foti *et. al.*, ٢٠٠٢) who revealed that primary infection with human cytomegalovirus (HCMV) is followed by persistence of the virus in a latent form. During life, the virus can be reactivated due to many factors including stress, disease, physiological or hormonal disturbance resulting in renewed shedding of the CMV or development of disease, which results in a possible lesion in the GIT.

The normal control people who had shown some level of specific anti CMV IgG antibody and had no peptic ulcers may be related to the infection with CMV in the previous months but there were no reactivation from the latent stage.

٥-٢-Anti CMV antibody Titers in Peptic Ulcer Patients According to Sexes

The results of the present work show that the incidence of peptic ulcer in males was higher than in females in different age groups. These results were approximately similar with (White ١٩٥١; Doll, ١٩٥٢; Clark, ١٩٥٣; Taha *et. al.*, ١٩٩٤; Oguta, ١٩٩٨; Robbins, ٢٠٠٠). The reason behind that may be that males exposure to stress more than females . Since the CMV is an opportunistic virus characterized by latent infections and can be reactivated under stress, which depends on type of work, and responsibility which is more complicated and harder for males than females. Similar findings were obtained by Beekhuis and Karrenbeld, ١٩٩٧. Moreover, the embargo on Iraq from ١٩٩٠ to ٢٠٠٣ and the recent conditions after the ٢٠٠٣ war on this country have had a significant effect on the level of services and increased stress which led to high occurrence of this diseases has been given by Al-Jawher, ١٩٩٧. (Figure ٤-٥) shows that only ٨ males and ٣ females out of ١٠٠ patients have had positive anti CMV antibodies in age groups from ١٦-٤٠ years old which represent the acute state of CMV infection.

٥-٣-Residential Distribution of Peptic ulcer Patients

Figure (٤-٦) shows that the rural resident's peptic ulcer patients were ٣٦% while urban resident's patients were ٦٤%. The number of peptic ulcer patients in urban area is significantly higher than that in rural area. Robbins (٢٠٠٠) revealed that the geographical area has a significant effect on the number of peptic ulcer patients and average of peptic ulcer infection varied from country to another and from region to another in the same country. Several reasons may reside behind that variations such as the population is crowded in urban area compared to rural area and this

increases the possibility of CMV infection, which has a major role in peptic ulcer disease. In addition, pollution, complicated lifestyle, using processed food and stress in urban area are more than rural area. The findings of the present work agreed with Sleisenger and Foerdtran (١٩٩٧) who revealed that urban residents are more exposed to peptic ulcer than rural residents because the urban residents may use processed and stored food, which might contain ulcerogens.

٥-٤-Histopathological Changes and Fluorescent Technique

Histopathological changes were considered as parameters indicative of the specific CMV proteins in the tissue sections. In addition to the pathological changes giant cell formation which is a main indication due to its relation with the presence of CMV in site of peptic ulcers. This method had been used by several scientists. Katlama (١٩٩٣) revealed that the diagnosis of CMV disease should be assessed on the association of clinical symptoms with the presence of inclusions in biopsy specimens. The histological examination of biopsies from ulcer regions showed presence of Cytomegalovirus inclusion bodies (Iwasaki,١٩٨٧; Forne *et. al.,* ١٩٨٩; Arnar *et. al.,* ١٩٩١; Chu *et.al*., ١٩٩٤ ; Chiu, ٢٠٠٤). Cheung and Ng IO (١٩٩٣) observed histological change in the epithelial distribution on The CMV inclusion bodies which was associated with no or only inflammation, whereas a predominant endothelial distribution of the inclusion bodies was associated with ulcerative, severely inflamed lesions. Yokose *et.*

al., ١٩٩٥ and Patra *et. al.,* ١٩٩٩ found histologically intranuclear CMV inclusions in biopsy specimens. Fluorescent microscope was used to determine the presence of CMV inclusion in biopsy specimens as similar method was used by Bruno *et. al*., (٢٠٠٥).

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Appendices

Appendices

A. The O.D. values and IgG, IgM level in ELISA IU/ml of all subjects in this study:

١. The O.D. values and IgG, IgM level of the patients.

No.	O.D.	IgG (IU/ml)	O.D.	IgM (IU/ml)
١	٠.٩٤٦	٢.٤٧٠	٠.٥٩٤	١.١٧٠
٢	٠.٧٠١	١.٨٣٠	٠.٥٣٨	١.٠٦٠
٣	٠.٨٤٣	٢.٢٠٠	١.٠٠٦	١.٩٨٠
٤	٠.٥٢١	١.٣٦٠	٠.٣٠٠	٠.٥٩٠
٥	٠.٨٨١	٢.٣٠٠	٠.٣٦٦	٠.٧٢٠
٦	٠.٧١٢	١.٨٦٠	٠.٣٦٦	٠.٧٢٠
٧	٠.٨٣١	٢.١٧٠	٠.٤٥٢	٠.٨٩٠
٨	٠.٦٩٣	١.٨١٠	٠.٣٨١	٠.٧٥٠
٩	٠.٦٤٧	١.٦٩٠	٠.٢٤٩	٠.٤٩٠
١٠	٠.٦٥٩	١.٧٢٠	٠.٣٩٦	٠.٧٨٠
١١	٠.٧٧٧	٢.٠٣٠	٠.٤٤٧	٠.٨٨٠
١٢	٠.٦٧٥	١.٧٦٤	٠.٢٨٢	٠.٥٥٥
١٣	٠.٦٠٩	١.٥٩٠	٠.٣٦٦	٠.٧٢٠
١٤	٠.٨٧٧	٢.٢٩٠	٠.٢٨٤	٠.٥٦٠
١٥	٠.٦٩١	١.٨٠٣	٠.٣٣٦	٠.٦٦١
١٦	٠.٦٨٠	١.٧٧٥	٠.٢٩٨	٠.٥٨٧
١٧	٠.٦٨٧	١.٧٩٣	٠.٣٦٥	٠.٧١٩

No.	O.D.	IgG (IU/ml)	O.D.	IgM (IU/ml)
١٨	٠.٨٧٣	٢.٢٧٩	٠.٣٣٨	٠.٦٦٥
١٩	٠.٦٤٠	١.٦٧٠	٠.٥٩٧	١.١٧٥
٢٠	٠.٦٤٦	١.٦٨٨	٠.٥٣٨	١.٠٥٩
٢١	٠.٦٥٣	١.٧٠٥	٠.٣٢٠	٠.٦٣١
٢٢	٠.٩٥٢	٢.٤٨٧	٠.٣٢٣	٠.٦٣٥
٢٣	٠.٦٦٦	١.٧٤٠	٠.٣٢٥	٠.٦٣٩
٢٤	٠.٦٧٣	١.٧٥٨	٠.٣٢٧	٠.٦٤٣
٢٥	٠.٥٩٩	١.٥٦٥	٠.٣٢٩	٠.٦٤٨
٢٦	٠.٨٨١	٢.٣٠٠	٠.٤٥٠	٠.٨٨٦
٢٧	٠.٦١٣	١.٦٠٠	٠.٣١٢	٠.٦١٤
٢٨	٠.٦٢٠	١.٦١٨	٠.٣١٤	٠.٦١٨
٢٩	٠.٦٢٦	١.٦٣٥	٠.٣١٦	٠.٦٢٢
٣٠	٠.٦٣٣	١.٦٥٣	٠.٣١٨	٠.٦٢٦
٣١	٢.٣١٦	٢.١٥٤	٠.٧٠٨	٠.٥٢٥
٣٢	٢.١١٧	١.٩٦٩	٠.٧٤٢	٠.٥٥٠
٣٣	١.٨٩١	١.٧٥٩	١.٠٧٩	٠.٨٠٠
٣٤	٢.٢٠٧	٢.٠٥٣	١.٣٨٤	١.٠٢٦
٣٥	٢.٣٦٢	٢.١٩٧	١.٥٤٢	١.١٤٣
٣٦	١.٨٢٣	١.٦٩٦	٠.١٦٢	٠.١٢٠
٣٧	٢.٦٣٣	٢.٤٤٩	٠.٥١٥	٠.٣٨٢
٣٨	٢.١٧٤	٢.٠٢٢	٠.٤٣٢	٠.٣٢٠
٣٩	٢.٤٧٨	٢.٣٠٥	٠.٧٢٤	٠.٥٣٧
٤٠	١.٦٩١	١.٥٧٣	٠.٣٦٤	٠.٢٧٠
٤١	٢.١٢٧	١.٩٧٩	١.٠٥٩	٠.٧٨٥
٤٢	١.٨٨٤	١.٧٥٣	١.٠٢٠	٠.٧٥٦
٤٣	٢.٥٥٧	٢.٣٧٩	٢.٩٨٤	٢.٢١٢
٤٤	٢.٦٧٧	٢.٤٩٠	٠.٤٤٧	٠.٣٣١
٤٥	٢.٤٤٢	٢.٢٧٢	١.٣٩٢	١.٠٣٢

No.	O.D.	IgG (IU/ml)	O.D.	IgM (IU/ml)
٤٦	١.٣٧٣	١.٢٧٧	٠.٣٧٦	٠.٢٧٩
٤٧	٢.٩٥٩	٢.٧٥٣	١.٤١٩	١.٠٥٢
٤٨	٢.٩٥٩	٢.٧٥٣	٠.٣٨٢	٠.٢٨٣
٤٩	١.٩٣٥	١.٨٠٠	٠.٥٣٠	٠.٣٩٣
٥٠	٢.٥٤٠	٢.٣٦٣	٠.٦٢٢	٠.٤٦١
٥١	٢.١٣٠	١.٩٨١	١.٠٩٠	٠.٨٠٨
٥٢	٢.٣٥٢	٢.١٨٨	١.١٩٨	٠.٨٨٨
٥٣	١.٨١٨	١.٦٩١	١.٣٧٢	١.٠١٧
٥٤	٢.٣٣١	٢.١٦٨	٠.٣٧٤	٠.٢٧٧
٥٥	٢.٢٣١	٢.٠٧٦	٠.٩٤٤	٠.٦٩٩
٥٦	٢.٢٣٥	٢.٠٧٩	٠.٩٤٧	٠.٧٠٢
٥٧	١.٥٣١	١.٤٢٤	٠.٩٥١	٠.٧٠٥
٥٨	٢.٢٤١	٢.٠٨٥	٠.٩٥٤	٠.٧٠٧
٥٩	٢.٢٤٥	٢.٠٨٨	٠.٩٥٧	٠.٧١٠
٦٠	٢.٢٤٨	٢.٠٩١	٠.٩٦١	٠.٧١٢
٦١	٢.٢٥١	٢.٠٩٤	٠.٩٦٤	٠.٧١٥
٦٢	٢.٢٥٥	٢.٠٩٧	٠.٩٦٨	٠.٧١٨
٦٣	١.٨٩٠	١.٧٥٨	٠.٩٧١	٠.٧٢٠
٦٤	٢.٢٦٢	٢.١٠٤	٠.٩٧٥	٠.٧٢٣
٦٥	٢.٢٦٥	٢.١٠٧	٠.٩٧٨	٠.٧٢٥
٦٦	٢.٢٦٨	٢.١١٠	٠.٩٨٢	٠.٧٢٨
٦٧	٢.٢٧٢	٢.١١٣	٠.٩٨٥	٠.٧٣٠
٦٨	١.٤٧٣	١.٣٧٠	٠.٩٨٩	٠.٧٣٣
٦٩	٢.٢٧٨	٢.١١٩	٠.٩٩٢	٠.٧٣٦
٧٠	٢.٢٨٢	٢.١٢٣	٠.٩٩٦	٠.٧٣٨
٧١	٢.٢٨٥	٢.١٢٦	٠.٩٩٩	٠.٧٤١
٧٢	٢.٢٨٩	٢.١٢٩	١.٠٠٣	٠.٧٤٣
٧٣	٢.٢٩٢	٢.١٣٢	١.٠٠٦	٠.٧٤٦

No.	O.D.	IgG (IU/ml)	O.D.	IgM (IU/ml)
٧٤	١.٩٩٩	١.٨٦٠	٠.٧٣٢	٠.٥٤٣
٧٥	٢.٢٩٩	٢.١٣٨	١.٠١٣	٠.٧٥١
٧٦	١.٧٥٦	١.٦٣٣	٠.٦٣٠	٠.٤٦٧
٧٧	٢.٣٠٥	٢.١٤٥	١.٠٢٠	٠.٧٥٦
٧٨	٢.٣٠٩	٢.١٤٨	١.٠٢٤	٠.٧٥٩
٧٩	٢.٣١٢	٢.١٥١	١.٠٢٧	٠.٧٦١
٨٠	١.٧٩٣	١.٦٦٨	٠.٣٢٤	٠.٢٤٠
٨١	٢.٣١٩	٢.١٥٧	١.٠٣٤	٠.٧٦٧
٨٢	٢.٣٢٢	٢.١٦٠	١.٠٣٨	٠.٧٦٩
٨٣	٢.٣٢٦	٢.١٦٣	١.٠٤١	٠.٧٧٢
٨٤	٢.٣٢٩	٢.١٦٦	١.٠٤٥	٠.٧٧٤
٨٥	١.٣٣٦	١.٢٤٣	٠.٤٥٦	٠.٣٣٨
٨٦	٢.٣٣٦	٢.١٧٣	١.٠٥٢	٠.٧٧٩
٨٧	٢.٣٣٩	٢.١٧٦	١.٠٥٥	٠.٧٨٢
٨٨	٢.٣٤٢	٢.١٧٩	١.٠٥٨	٠.٧٨٥
٨٩	١.٩٥٣	١.٨١٧	٠.٥٢٣	٠.٣٨٨
٩٠	٢.٣٤٩	٢.١٨٥	١.٠٦٥	٠.٧٩٠
٩١	٢.٣٥٣	٢.١٨٨	١.٠٦٩	٠.٧٩٢
٩٢	٢.٣٥٦	٢.١٩٢	١.٠٧٢	٠.٧٩٥
٩٣	١.٣٥٦	١.٢٦١	٠.٣٤٧	٠.٢٥٧
٩٤	٢.٣٦٣	٢.١٩٨	١.٠٧٩	٠.٨٠٠
٩٥	٢.٣٦٦	٢.٢٠١	١.٠٨٣	٠.٨٠٣
٩٦	١.٣٦٩	١.٢٧٣	٠.٢٥٤	٠.١٨٨
٩٧	٢.٣٧٣	٢.٢٠٧	١.٠٩٠	٠.٨٠٨
٩٨	٢.٣٧٦	٢.٢١٠	١.٠٩٣	٠.٨١٠
٩٩	١.٣٨٤	١.٢٨٧	٠.٤٤٦	٠.٣٣١
١٠٠	٢.٣٨٣	٢.٢١٧	١.١٠٠	٠.٨١٦

٢. The O.D. values and IgG, IgM level of the normal people.

No.	O.D	IgG (IU/ml)	O.D.	IgM (IU/ml)
١	١.٥٨	١.٤٧٢	١.١٦٦	٠.٨٦٤
٢	١.٦٥	١.٥٣٩	٠.٨٧١	٠.٦٤٦
٣	٠.٩٧	٠.٩٠٥	٠.٥٤٢	٠.٤٠٢
٤	٠.٦٨	٠.٦٣٧	٠.٣٧٢	٠.٢٧٦
٥	١.١٨	١.٠٩٨	٠.٧١٥	٠.٥٣
٦	٠.٣٥	٠.٣٢٨	١.٠٢٨	٠.٧٦٢
٧	١.٣٨	١.٢٨٧	٠.٥٥٨	٠.٤١٤
٨	٠.٧٥	٠.٦٩٧	٠.٦٥٦	٠.٤٨٦
٩	٠.٣٥	٠.٣٢٦	٠.١٨٩	٠.١٤
١٠	٠.٨١	٠.٧٥٣	٠.٦٩٦	٠.٥١٦
١١	٠.٩٣	٠.٨٦٤	١.٢١١	٠.٨٩٨
١٢	٠.٧٣	٠.٦٧٨	٠.٦٠٣	٠.٤٤٧
١٣	٠.٧٣	٠.٦٨٢	٠.٦٣٣	٠.٤٦٩
١٤	٠.٩٦	٠.٨٩٧	١.٠٢٨	٠.٧٦٢
١٥	٠.٧٨	٠.٧٢١	٠.٧٧٨	٠.٥٧٧
١٦	٠.٨٢	٠.٧٦٢	٠.٥٢٦	٠.٣٩
١٧	٠.٧٠	٠.٦٥٢	٠.٧٣٠	٠.٥٤١
١٨	٠.٨٤	٠.٧٨٦	٠.٣٩٣	٠.٢٩١
١٩	٠.٧٦	٠.٧٠٩	٠.٩٩٦	٠.٧٣٨
٢٠	٠.٥٩	٠.٥٤٨	٠.٣٧٤	٠.٢٧٧
٢١	٠.٨٦	٠.٧٩٦	٠.٦١٦	٠.٤٥٧
٢٢	٠.٧٦	٠.٧١	٠.٤٤٠	٠.٣٢٦
٢٣	٠.٦٦	٠.٦١	٠.٢٧٧	٠.٢٠٥
٢٤	٠.٦٤	٠.٥٩٥	١.١٥٢	٠.٨٥٤
٢٥	٠.٣٤	٠.٣٢	٠.٦٤٩	٠.٤٨١
٢٦	٠.٥٩	٠.٥٤٨	١.٠٣١	٠.٧٦٤
٢٧	٠.٨٦	٠.٧٩٦	١.١٧٩	٠.٨٧٤

No.	O.D	IgG (IU/ml)	O.D.	IgM (IU/ml)
٢٨	٠.٧٦	٠.٧١	١.١٤٠	٠.٨٤٥
٢٩	٠.٦٨	٠.٦٣٧	٠.٣٧٢	٠.٢٧٦
٣٠	٠.٦٩	٠.٦٤٢	٠.٧١٥	٠.٥٣
٣١	٠.٣٥	٠.٣٢٨	١.٠٢٨	٠.٧٦٢
٣٢	١.٣٨	١.٢٨٧	٠.٥٥٨	٠.٤١٤
٣٣	٠.٧٥	٠.٦٩٧	٠.٦٥٦	٠.٤٨٦
٣٤	٠.٢٠	٠.١٨٩	٠.١٨٩	٠.١٤
٣٥	٠.٦٩	٠.٦٤٣	٠.٦٩٦	٠.٥١٦
٣٦	٠.٩٥	٠.٨٨٥	١.١٤٩	٠.٨٥٢
٣٧	٠.٧٩	٠.٧٣٩	٠.٦٠٣	٠.٤٤٧
٣٨	٠.٧٣	٠.٦٨٢	٠.٦٣٣	٠.٤٦٩
٣٩	١.٩٢	١.٧٨٥	٠.٧٥٩	٠.٥٦٣
٤٠	٠.٧٧	٠.٧١٤	٠.٧٧٨	٠.٥٧٧
٤١	٠.٨٢	٠.٧٦٢	٠.٥٢٦	٠.٣٩
٤٢	٠.٦٩	٠.٦٤١	٠.٧٣٠	٠.٥٤١
٤٣	٠.٥٠	٠.٤٦٣	٠.٣٩٣	٠.٢٩١
٤٤	٠.٥٦	٠.٥١٧	٠.٤٤٠	٠.٣٢٦
٤٥	٠.٦٦	٠.٦١	٠.٢٧٧	٠.٢٠٥
٤٦	٠.٦٤	٠.٥٩٥	١.١٥٢	٠.٨٥٤
٤٧	٠.٣٤	٠.٣٢	٠.٦٤٩	٠.٤٨١
٤٨	٠.٥٩	٠.٥٤٨	٠.٥٨١	٠.٤٣١
٤٩	٠.٨٠	٠.٧٤٦	٠.٨٧١	٠.٦٤٦
٥٠	٠.٩٧	٠.٨٩٨	٠.٥٤٢	٠.٤٠٢

B. Optical density of cutoff, positive and negative calibrators.

CMV IgG(IU/ml)	O.D.
٠	٠.٠٥٨
١.٢	١.٠٧٥
٦	١.٩٣١
١٨	٢.٣٥٨