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STUDY OF CO-EFFECT OF *HELICOBACTER PYLORI* ON PREGNANT WOMEN INFECTED WITH CYTOMEGALOVIRUS

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ABSTRACT

Background Human cytomegalovirus (CMV) is a member of the herpesvirus family. Though CMV may found in many organs, cytopathogenicity observed infrequently and clinical manifestations of CMV in the immunocompetent host are rare some of cases infected with CMV and *Helicobacter pylori* co-infection. **Material and methods:** the total cases were showed (165) pregnant women which IgG positive collected from Al-Azhar Teaching Hospital (Sayed Glal) Cairo, Egypt; CMV was detected of by Enzyme-linked immunosorbent assays (ElISA), and real time polymerase chain reaction (rt-PCR), the relation between CMV and *Helicobacter pylori* with risk factor (age, time of apportion, number of apportion, number of delivery, life style, Blood transfusion, use of corticosteroids, gynecologic and medical history of abortion, residence and socioeconomic status). **Result:** Positive of CMV and H. pylori infected pregnant women were **128** samples as **(77%),** All samples IgG positive (CMV) in serological tests, All samples negative for (TORCH) Toxoplasmosis Rubella and Herpes simplex virus (HSV), when study Co-effect between CMV and H. pylori found significant positive correlation between PCR, age patients, number of abortion, and. While negative correlation between PCR time of abortion, number of delivery, Use of corticosteroids, Blood Transfusion and life style, there was no statistical significant correlation between PCR and Birth Defect.

KEYWORDS: CMV, ElISA, rt-PCR, Virus, Risk factor, Abortion, pregnant women, *H. pylori*.

INTRODUCTION

In Korea, Iwama present the first a case of pediatric Ménétrier's disease with positive evidence of CMV and *H. pylori*. **(Iwama et al., 2010)** Cytomegalovirus (CMV) identified as a virus in the 1950's after collaboration between Weller, Rowe and Smith that independently isolated the virus from patient samples. CMV typically infects cells of the myeloid lineage along with epithelial and endothelial cells **(Ho, 2008).**

In 1956, CMV was isolated for the first time and the true nature of this infectious agent became clear. Weller gave the virus its name from the cytopathic effect produced in cell Culture With the advent of advanced cell culture techniques, the virus causing these cytomegalic cells with intranuclear inclusions was isolated, and hence named Cytomegalovirus **(Craig *et al.*, 1957).** Human cytomegalovirus (HCMV) is a human beta herpes virus Members of the Herpesviridae family **(Crough and Khanna, 2009).**

(Chakravarti et al., 2007) mention that One of previous studies shows that an IgG avidity assay could be used in combination with an IgM ELISA for monitoring pregnant women for primary CMV Infection. **(Lequin, 2005)** refer that Enzyme-linked immunosorbent assay (ELISA), is a popular format of a "wet-lab" type analytic biochemistry assay that uses one sub-type of heterogeneous, solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antibodies, in a liquid sample or wet sample. This study aims to evaluation the best methods for detection CMV in pregnant women to right diagnoses and treatment. (CMV) is one of the major causes of congenital infections. Its clinical manifestations range from asymptomatic forms (90% of cases) to severe fetal damage and, in rare cases, death due to abortion. Furthermore, 10%–15% of the children who are asymptomatic at birth may develop late squeal, especially hearing defects, after a period of months or even years **(Stagno and Whitley 1985)** The risk of fetal damage is greater if the primary infection occurs during the first trimester of pregnancy **(Stagno *et al.*, 1986; Adler, and Marshall, 2007),** the prevalence of congenital infection ranges from 0.2% to 2.5% in different populations **(Demmler, 1991; Barbi *et al.*, 1998).**

Furthermore, the prevalence of congenital infection varies with the prevalence of the infection in the population **(Malm, and Engman, 2007)** as well as increasing with age, may also depend on sexual activity and occupation, particularly occupations involving close contacts with children in a community setting, In the case of parents, contact with the urine or

saliva of their children is a major source of infection **(Pass *et al.*, 1986; Fowler and Pass 2006).** The risk of acquiring infection during pregnancy is 0.7–1.38 % **(Staras, *et al.*, 2006).**

This study Aimed to study relationship between CMV and *H. pylori*, and evaluate the best detection method for diagnosis CMV.

MATERIALS AND METHODS

Assessment of CMV in serum blood cases carried out by Serological Methods and molecular methods rt-PCR, total CMV DNA. This study carried out on one hundred and sixty five patients pregnant woman (PW) with repeated Abortion happened more than once before pregnant, and diagnosis as CMV IgG positive using Rapid test and ELISAs for CMV antigen in serum. Take Whole blood (5ml) obtained under aseptic conditions from each subject by a vein puncture using a disposable syringe. Blood samples divided into two tubes. First tube: EDTA tubes for viral DNA isolation. Second tube: The serum was obtained by putting the blood samples in a clean dry plain plastic tube and was allowed to clot at 37 ºC for 30 minutes before centrifugation. The tubes were centrifuged at 2000 rpm for 20 minutes, take supernatant (Serum) for serology test (CMV and *H. pylori*), chemical that analyzed at once (LH, FSH, and Prolactin), A structured interview using a standard maternal questionnaire administered by trained interviewers with the women at their first visit. Questions were asked about the following: age, time of apportion, number of apportion, number of delivery, life style, Blood transfusion, use of corticosteroids , gynecologic and medical history of abortion, residence and socioeconomic status. The ELISA technique performed using kits intended for estimating concentration of specific CMV-IgM and CMV-IgG markers. The kits were purchased from Sigma Diagnostics (USA), the techniques were performed according to the manufacturer's instructions.

CMV ELISA assay procedure according to Sigma Aldrich ® CMV IgG EIA test Kit. The Sigma Aldrich ® CMV IgG EIA test Kit, (Sigma Aldrich, USA, Cat. No: 1311) is an in vitro diagnostic kit designed for the detection of cytomegalovirus (CMV) IgG in human blood serum As recommended of **(Nielsen 1988)**.

Real-Time PCR Assay

Real-Time PCR assay procedure according to AccuPower® CMV Quantitative PCR Kit. The AccuPower® CMV Quantitative PCR Kit (Bioneer, South Korea, Cat. No: 1111) is an in

vitro diagnostic kit designed for the quantification of cytomegalovirus (CMV) DNA in human blood serum.

Nucleic acid was extracted from 0.2 ml of each specimen by using AccuPower® CMV Quantitative PCR Kit. blood kit according to the manufacturer's protocol. DNA was eluted from the column with 50 µl of PCR-grade H2O. This procedure is also suitable for the extraction of viral DNA from urine because of the high DNA yield and the removal of PCR inhibitors (4). An aliquot of 5 µl of the extracted nucleic acid was added to 15 µl of reaction mixture containing 4 mM MgCl2, a 0.66 µM concentration of each primer for the glycoprotein B gene, 0.4 µM fluorescein hybridization probe (TIB MOLBIOL, Berlin, Germany), 0.4 µM LC-Red 640 probe (TIB MOLBIOL), 5% formamide, and 2 µl of Light Cycler Fast Start DNA (Master Hybridization Probes kit; Roche Molecular Biochemical, Mannheim, Germany). The hybridization probe sequences (5′ to 3′ direction) were as follows: donor fluorophore probe, CGTTTCGTCGTAGCTACGCRTACAT-fluorescein; acceptor fluorophore probe, LC-Red 640-ACACCACTTATCTYCTGGGCAGC-phosphate. Reaction capillaries were loaded, centrifuged, and placed into the carousel of a Light Cycler instrument (Roche Molecular Bio chemicals). The experimental PCR protocol was as follows: an initial 10 min at 95°C for Fast Start Taq DNA polymerase activation, followed by 45 cycles of 10-s denaturation at 95°C, 15-s annealing at 58°C, and 12-s extension at 72°C. Data were obtained during the annealing period in the "single" mode, with the channel setting F2/F1. Fluorescence settings were as follows: F1 gain, 1; F2 gain, 14; and F3 gain, 10. The specificity of the obtained fluorescence signal was checked by a melting-curve analysis after each run. This analysis was initiated at a temperature of 45°C, which was gradually raised. During this process, the fluorescence signal was continuously monitored. The melting temperature of the specific probes was 59.2°C. The primer used is GTACACGCACGCTGGTTACC Sequence (5′→ 3′) Target region TRL11.

Helicobacter pylori (*H. Pylori*) assays were done by (ELISA) using the kits supplied by Diagnostic Products Corporation, USA. According to **(Marshall, et al., 1984; Goodwin, *et al.*, 1987).**

The hormonal assays (LH, FSH, and Prolactin) were done by (ELISA) using the kits supplied by Diagnostic Products Corporation, USA. According to **(Odell and Parlow 1981)** Normal levels of FSH is 3-20 mIU/ml, LH< 7 mIU/ml, Prolactin< 24 ng/ml.

Statically analysis: For assessment of risk factors for CMV infection (exposure), characteristics of case patients and control subjects examined using a two-sample Student t test. Cross-tabulation and chi-square or Fisher exacts tests were used to examine the relationship between variables using a 95% confidence interval as a measure of association **(Fisher, *et al.*, 2000).** The sample size was calculated according to Raosoft and all statistical calculations were done using SPSS (statistical package for the social science version 20.00) statistical program at 0.05 level of probability **(Snedecor and Cochran, 1982).**

RESULTS

This study was done on 165 pregnant female patients. Their ages ranged from 20 to 41 years (mean of 26.37±0.34 SE). They were Time of abortion, number of abortion and number of delivery ranged from 1 to 3, 0-2, 0-3 respectively (mean of 2.16±0.06, 1.19±0.03, 1.042±0.05 respectively).

In addition, 70 patients village of life style representing 42.4% of female patients and 95 female city of life style representing 47.6% of female patients, 67 females Use of corticosteroids representing 40.6% and 98 females don't Use of corticosteroids representing 59.4% and 27 females Blood Transfusion representing 16.4% and 138 females don't Blood Transfusion.

Table (1): Correlation between PCR in relation to Rapid chromatographic Immunoassay, ELISA.

	H pylori	
	Correlation coefficient (r)	**P-value**
PCR (CMV)	0.929**	0.000
ELISA (CMV)	0.942**	0.000

** Correlation is significant at the 0.01.

Showed a statistical significant negative correlation between PCR, Rapid chromatographic Immunoassay, While positive correlation between PCR, ELISA, as shown in table (1), and figures (1, and 2).

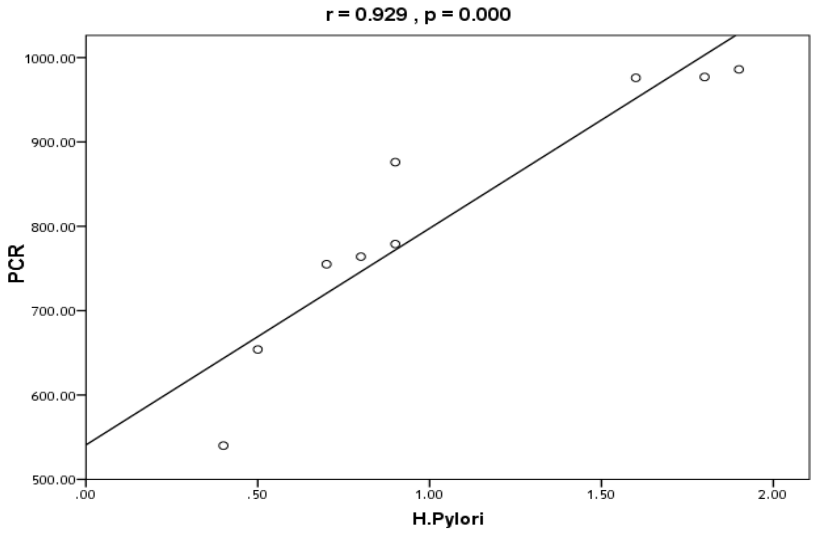

Figure (1): Linear correlation between PCR, H. pylori and their statistical significance.

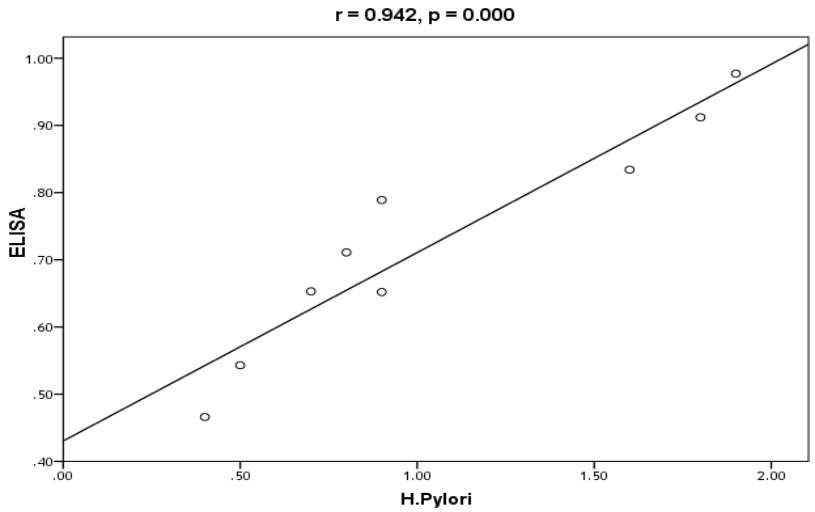

Figure (2): Linear correlation between ELISA, H. pylori and their statistical significance.

Table (2): Comparison between Life style as regard co-infection between H. Pylori and CMV.

	Life style		Z test	P value
	Village N = 128	City N =37		
(H. Pylori / CMV)	1.23±0.19	0.45± 0.50	2.058	0.040 S

S: Significant at P value ≤ 0.05

Co-infection between H. Pylori and CMV levels showed a significant decrease in Village life style patients that mean ± SE 1.23±0.19 when compared with city life style that mean ± SE 0.45± 0.50 (P value = 0.040) as shown in table (2), and figure (3).

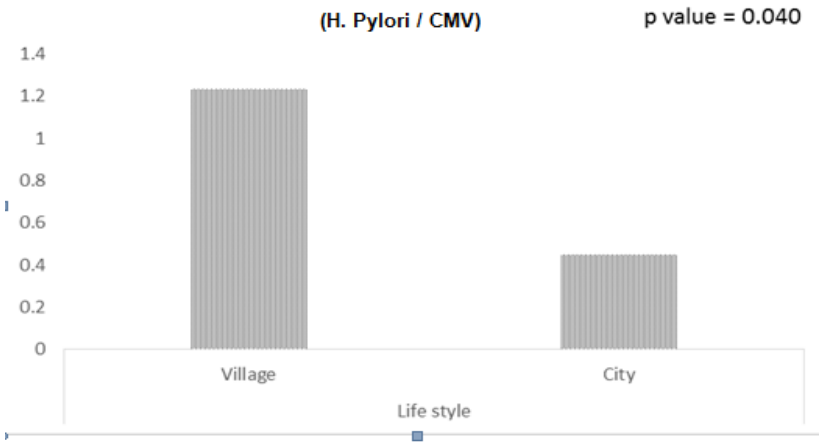

Figure (3): Comparison between both groups as Co-infection between H. Pylori, CMV and their statistical significance.

Table (3): Comparison between Uses of corticosteroids as regard Co-infection between H. Pylori, CMV.

	Use of corticosteroids		Z test	P value
	Yes N = 128	No N =37		
(H. Pylori / CMV)	1.23±0.19	0.45± 0.50	2.058	0.040 S

S: Significant at P value ≤ 0.05

Co-infection between H. Pylori and CMV levels showed a significant decrease in Use of corticosteroid patients that mean ± SE 1.23±0.19 when compared with don't Use of corticosteroid that mean ± SE 0.45± 0.50 (P value = 0.040) as shown in table (3), and figure (4).

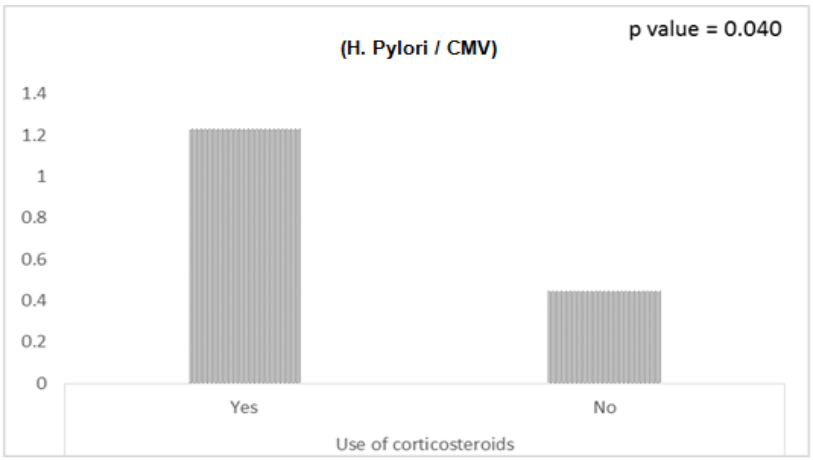

Figure (4): Comparison between both groups as H. Pylori and their statistical significance.

Table (4): Comparison between blood transfusion as regard H. Pylori.

	Blood Transfusion		t test	P value
	Yes N = 73	No N =92		
(H. Pylori / CMV)	1.55±0.22	0.66± 0.09	3.969	0.005 S

S: Significant at P value ≤ 0.05

Co-infection between H. Pylori and CMV levels showed a significant decrease in Blood Transfusion patients that mean ± SE 1.55±0.22 when compared with don't Blood Transfusion that mean ± SE 0.66± 0.09 (P value = 0.005) as shown in table (4), and figure (5).

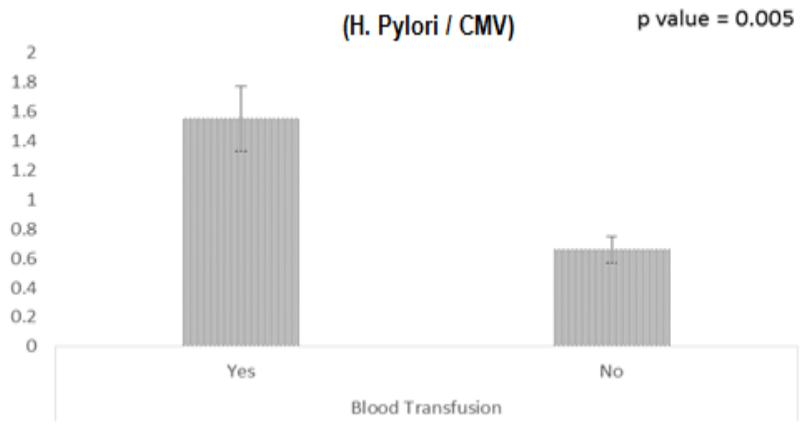

Figure (5): Comparison between both groups Blood Transfusion as H. Pylori and their statistical significance.

Table (5): Correlation between (Co-infection between H. Pylori and CMV levels) in relation to age patients, Time of abortion, number of abortion, number of delivery, Use of corticosteroids, Blood Transfusion, Birth Defect and life style.

	Co-infection between H. Pylori and CMV	
	Correlation coefficient (r)	P-value
Age	0.733*	0.025
Time of abortion	-0.657*	0.038
Number of abortion	0.598	0.089
Number of delivery	-0.650	0.058
Use of corticosteroids	-0.609	0.082
Blood Transfusion	-0.832**	0.005
Life style	-0.609	0.082

** Correlation is significant at the 0.01

Showed a statistical significant positive correlation between PCR, age patients, and number of abortion, while negative correlation between PCR, time of abortion, number of delivery, Use of corticosteroids, Blood Transfusion and life style. Otherwise, there were no statistical significant correlations between PCR, Birth Defect, as shown in table (5), and figures (6, 7, and 8).

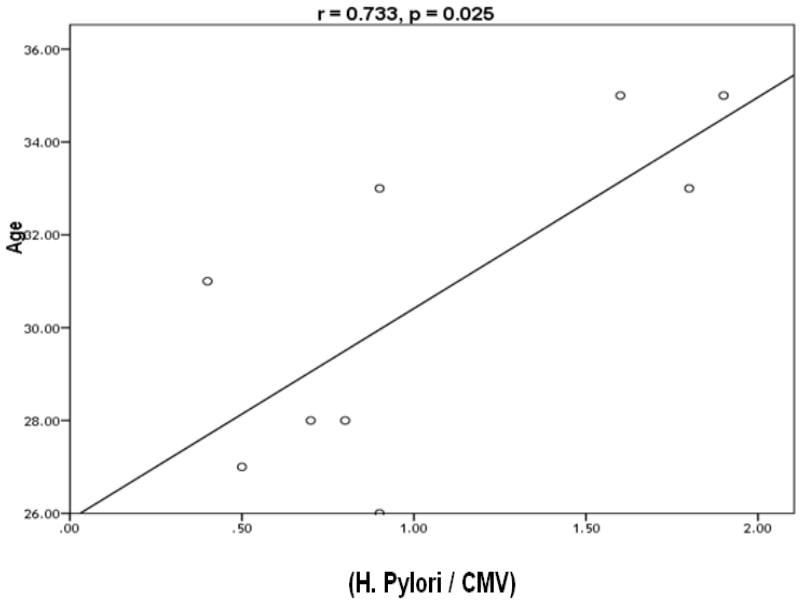

Figure (6): Linear correlation between Age, H. pylori and their statistical significance.

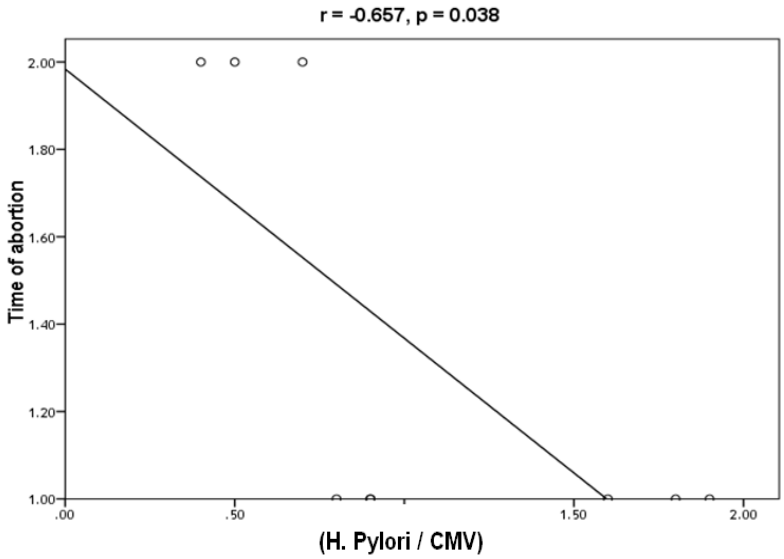

Figure (7): Linear correlation between time of abortion, H. pylori and their statistical significance.

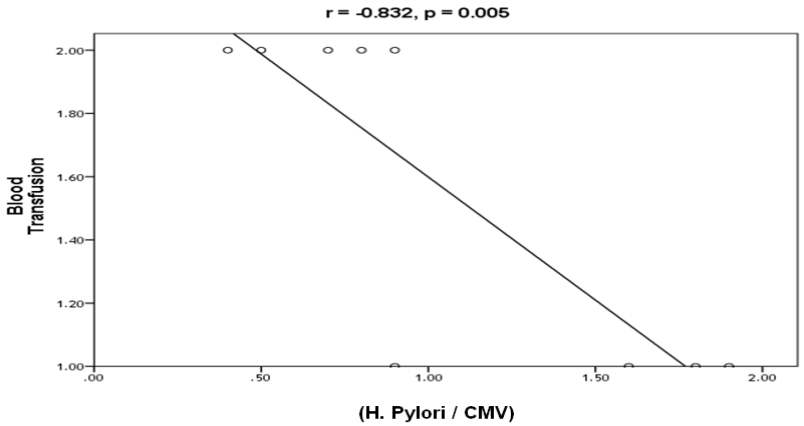

Figure (8): Linear correlation between Blood Transfusion, H. pylori and their statistical significance.

Table (6): Correlation between PCR in relation to FSH, LH, prolactin, Hb, WBCs.

	Co-infection between H. Pylori and CMV	
	Correlation coefficient (r)	**P-value**
FSH (mIU/ml)	0.466	0.206
LH (mIU/ml)	-0.281	0.464
Prolactin (ng/ml)	0.417	0.264
Hb (g/dl)	-0.839**	0.005
WBCs(cu.mm)	-0.845**	0.004

**. Correlation is significant at the 0.01.

Showed a statistical significant negative correlation between Co-infection between H. Pylori and CMV, Hb, WBCs. Otherwise, there were no statistical significant correlation between (Co-infection between H. Pylori and CMV), FSH, LH, and prolactin, as shown in table (6), and figures (9, and 10).

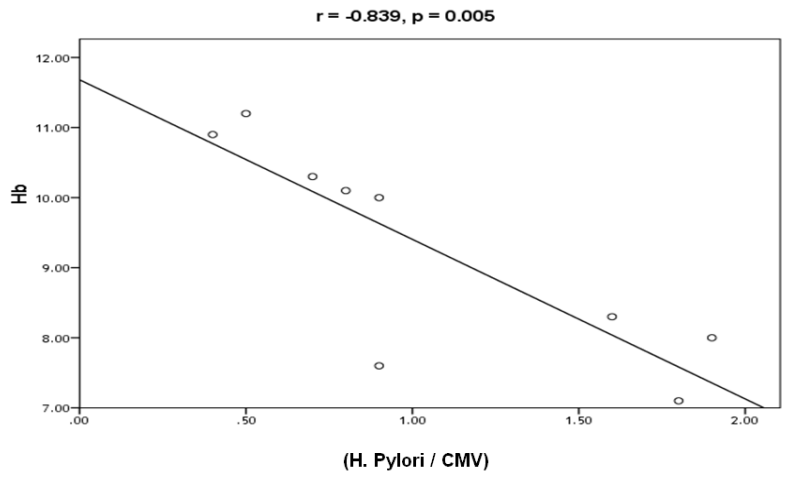

Figure (9): Linear correlation between Hb, H. pylori and their statistical significance.

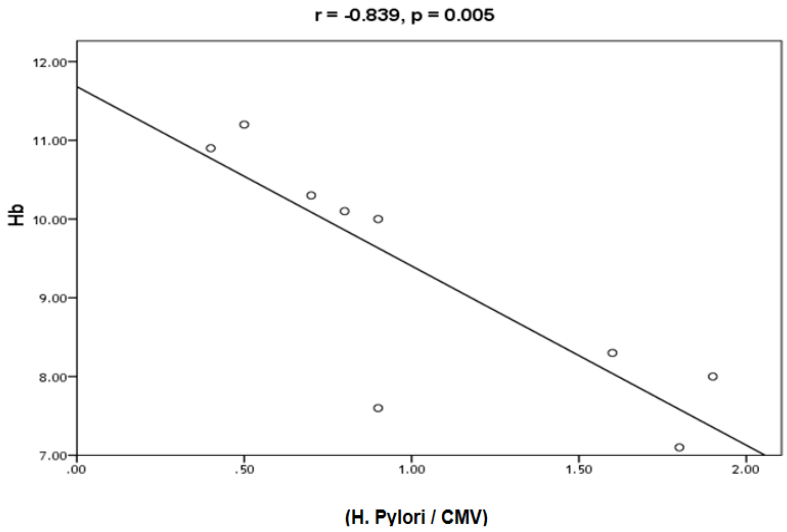

Figure (10): Linear correlation between, WBCs, H. pylori and their statistical significance.

DISCUSSION

Table (7) showed the results for both serological, Real-time tests.

Test performed	No. Positive		No. Negative	Reference range
Rapid test	IgG	165	0	Negative
	IgM	99 (60%)	66 (40%)	Negative
ELISA	IgG	165	0	Negative (> 0.345)* positive (< 0.345)
	IgM	132 (80%)	33 (20 %)	
Real-time PCR	158 (96%)		7 (4%)	Negative (≤350)** Positive (>350)

* = Sigma Aldrich ® CMV IgG EIA test Kit.

**= AccuPower® CMV Quantitative PCR Kit.

It turned out the results rt-PCR more efficient in detection of CMV as (96%), more than ELISA (80%), while rapid (60%). The rapid test CMV card is a rapid chromatographic immunoassay for the qualitative detection of CMV antigen in sample to aid in the diagnosis of CMV infection (IgG, IgM) **(Afifi *et al.*, 2009)**

Positive of CMV and H. pylori infected pregnant women were **128** samples as **(77%)** One of previous studies shows that an IgG avidity assay could be used in combination with an IgM ELISA for monitoring pregnant women for primary CMV Infection. The ELISA used as a diagnostic tool in medicine, as well as a quality-control check in various **(Chakravarti *et al.*, 2007)**.

Co-infection between H. Pylori and CMV levels showed a statistical significant positive correlation between PCR, ELISA, age patients, and number of abortion, while negative correlation between PCR, time of abortion, number of delivery, Use of corticosteroids, Blood Transfusion and life style **(Khudhur et al., 2014)** detected HCMV in 151 (70%) of the 214 abortion women by IgG and IgM antibody. According to the age of patients, No difference were seen in results. Also, **(Pouria *et al.*, 1998; Munro *et al.*, 2005; Arabpour *et al.*, 2007; and Tawfeq, 2009)** got same results, while **(Shams *et al.*, 2011)** noticed that there is a difference between results according to patient's age. In study by **(Al-Kazaz *et al.*, 2014)** HCMV detection by ELISA and Found 46 positive to IgG out of 46 and 40 positive to IgM out of 46. **(Ramadhan and Jihad, 2015)** reported that miscarriage women were highest percentage of seropositive to HCMV for IgG (40%) and (25%) for IgM out of 40 samples. **(Yasir, 2012)** showed higher of IgG positive at age (27-32) also **(Sotoodeh, 2009)** showed 94% of positive IgG at age (25-34). **(Shams *et al.*, 2011)** found from 327 pregnant women 54 were anti HCMV positive. Anti HCMV was detected in 94% of the women who had abortion history. **(Jalal, 2010; Edmunds, 2000)** conducted their study on 130 (aborted women, suspected men and pregnant women) could detect positive IgM to 70 patient and positive IgG to 97 patient. The prevalence rates of human cytomegalovirus IgM and IgG in non-pregnant women have been reported to be 1% and 84% respectively, and 2.5% and 90% in pregnant women **(Alwan 2011).**

Co-infection between H. Pylori and CMV levels showed a significant decrease in Village life style patients that mean ± SE 1.23±0.19 when compared with city life style that mean ± SE 0.45± 0.50, Use of corticosteroid patients that mean ± SE 1.23±0.19 when compared with don't Use of corticosteroid that mean ± SE 0.45± 0.50 (P value = 0.040) **(Khalf *et al.*, 2012)** who detected positive IgM to HCMV in 15.7 of 108 pregnant women. Also **(Mahdi *et al.*, 2011)** reported that an increase in seropositive HCMV IgG in relation with Village and city life style patients, Use of corticosteroid and don't Use of corticosteroid, The presence of a specific IgG antibody means that previous infection or acute infection with HCMV. This is considered true when there is no PCR product because IgG level over time is an uncertain approach for distinguishing primary from non-primary HCMV infection **(Prince, 2002).**

Otherwise, there were no statistical significant correlations between Co-infection between H. Pylori and CMV, and Birth Defect. A statistical significant negative correlation between Co-infection between H. Pylori and CMV, Hb, Rapid chromatographic Immunoassay, and

WBCs. **(Jahromi, 2010)** The presence of a specific IgM antibody means that the women are in primary infection, It is also produced during reactivation and reinfection. The prevalence rates of IgM antibody have been associated with different causes such as women in pregnant state because the primary HCMV infection has been found to be more rate in pregnant women than non-pregnant women this difference may be attributed to women with seronegative to HCMV more susceptibility at beginning of pregnancy to the first infection with HCMV **(Stagno, 1982),** that may because suppressed immune systems in pregnant women. Risk factors for HCMV infection have been correlated with the socioeconomic status within community. **(Fowler, 2003)** Real time PCR was rapid, sensitive and useful technique for diagnosis active disease and monitoring response to therapy **(Prince, 2002).**

Otherwise, there was no statistical significant correlation between Co-infection between H. Pylori and CMV, FSH, LH, and prolactin Miscarriage is the spontaneous loss of pregnancy between conception and 24 weeks into pregnancy. **(Goodrich, 2004)** Serum specimens were tested for the presence of IgM using enzymatic immunoassay technique all of them IgG positive. These included (165) women with previous history of recurrent abortions, intrauterine death (IUD), and premature deliveries.

(Occena *et al.*, 1993) reviewed reported pediatric disease cases and described the association with CMV in 70% (19 of 27) of cases. Subsequently, other reports describing CMV in association with *H. pylori* disease appeared (17 of 22, since 2008) **(Canan *et al.*, 2008).** Although the mechanism is not clearly understood, it has been reported in a few studies that TGF-α is immune reactively increased by CMV **(Megged *et al.*, 2008)**.

CMV infection usually gives signs in the gastric fundus and corpus and may lead to wall thickening, ulceration, hemorrhage, and perforation **(Yokose *et al.*, 1995).** Characteristic histologic symptoms include hypertrophy of the gastric mucosa associated with fever hyperplasia. In addition, symptoms such as hypertrophic gastric glands, interstitial inflammatory reaction, glandular atrophy, and cysts on mucous cells may also be observed. When we examined our patient, who was serologically diagnosed with positive CMV IgM, we detected edematous corpus, hyperemic mega-folds, and hemorrhagic gastritis by upper GIS endoscopy. Histopathologically, chronic pan-mucosal gastritis and, in tissue, CMV positive intraepithelial inclusion bodies were observed. It has been stated that the reason for this observation is that CMV is demonstrative in the early stages but cannot be seen in later stages **(Yokose et al. 1995).** Gastrointestinal CMV infection is mostly seen in patients with

immune deficiency. In these cases, it may affect a part of the gastrointestinal system or may cause a generalized infection. In immunodeficient patients, CMV infection is mostly seen in the colon, stomach, and esophagus, while in patients with normal immune systems, the stomach is most frequently affected **(Yokose et al. 1995; Patra et al. 1999)**. For diagnosis, CMV serology is vital during routine screening. Thus, compared to serology, detection of CMV DNA in biopsy material through PCR is a more sensitive method. However, since this method is expensive and cannot be performed everywhere, its application is limited. The recommended method for diagnosis is to perform serological tests routinely and to examine gastric biopsy material immune histochemically via PCR **(Megged and Schlesinger 2008)**

In adults, *H. pylori* is thought to have a role in gastric disease, rather than CMV **(Bayerdörffer et al. 1994).**

The proposed pathogenic mechanism is mucosal damage caused by infection with CMV or *H. pylori*, which may involve the production of abnormal local transforming growth factor-α, which in turn stimulates cell proliferation of gastric mucosa, inhibits gastric secretion, and enhances mucus secretion **(Faure et al. 1996).**

(Tokuhara et al. 2007) reported a case of pediatric gastric disease involving co-infection with CMV and *H. pylori*. After eradication therapy for *H. pylori*, the thickened gastric folds resolved. The authors concluded that this case of pediatric gastric disease was secondary to *H. pylori* infection rather than CMV infection.

(Iwama et al. 2010) reported another CMV and *H. pylori* co-infection in gastric disease case. They suggested that *H. pylori* had a causative role rather than CMV.

In these cases of pediatric in gastric disease involving co-infection with CMV and *H. pylori*, eradication of *H. pylori* contributed to improvement of not only endoscopy finding, but also clinical symptoms.[14] Because *H. pylori* was thought to be causative pathogen as well as CMV in our case, *H. pylori* eradication was tried. Although follow-up 13C urea breath test was not changed to negative, patient's symptoms was improved and the findings of esophagogastroduodenoscopy were normalized.

In summary, we describe another gastric disease with CMV and *H. pylori* co-infection, which is a very rare case in pregnant women. The present case information might be helpful for better understanding the nature and course of the disease.

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