

## Study the Impact of Cytomegalovirus (CMV) Infection and the Risk Factor for Liver Dysfunction in Saudi Patients

Randa Mohamed Ahmed Farag<sup>1</sup>, Dujana AlAyobi<sup>2</sup>, Khalid A Alsaleh<sup>3</sup>, Hye-Joo Kwon<sup>4</sup>, Afaf EL-Ansary<sup>5</sup> and Emad Anwar Dawoud<sup>6</sup>

<sup>1</sup>Assistant Prof of Virology and Molecular Biology, Health Sciences Research Center (HSCR), Princess Nourah bint Abdulrahman University (PNU), Kingdom of Saudi Arabia (KSA).

<sup>2</sup>Prof of Genetic, Biology Department, Princess Nourah bint Abdulrahman University (PNU), Kingdom of Saudi Arabia (KSA).

<sup>3</sup>Prof of Oncology and Hematology, college of Medicine, King Saud University (KSU), Kingdom of Saudi Arabia (KSA).

<sup>4</sup>Assistant Prof of Molecular Biology, Princess Nourah bint Abdulrahman University (PNU), Kingdom of Saudi Arabia (KSA).

<sup>5</sup>Prof Biochemistry, Central Lab, King Saud University (KSU).

<sup>6</sup>Assistant Prof of Hepatopathology, Faculty of Medicine, EL-Azher University and Specialist Physician, Oncology Clinic-Medical Affairs, Tawam Hospital, AL Ain, UAE.

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Human cytomegalovirus (CMV) is the leading infectious cause of congenital infection in developing countries. However, the pathogenesis of CMV infection is poorly understood. Worldwide, CMV seropositivity varies with age and sex, but data related to these variations are lacking. Here, we examined the variation in CMV seroprevalence in three different age groups of Saudi population in an attempt to understand the variation in the relationship between CMV infection seroprevalence with age and study the risk factor liver dysfunction. Serum samples for 455 patients with elevation of liver profiles (ALT, AST, ALP and GGT) were screened for CMV- IgG and IgM and categorized in different age groups. A correlation between CMV seropositivity measured with IgG and IgM and liver enzymes were tested. Receiver operating characteristics (ROC) analysis and multiple regressions were used for data analysis. Our study shows that young people (18-25 years) had much higher IgG and IgM compared to elderly people (26 -35 and 36-45 years). A significant correlation between both antibodies and liver enzymes (AST, ALT) was recorded. Receiver operating characteristics (ROC) analysis revealed that both IgG and IgM can be used as excellent predictive markers for CMV infection as both recorded 100% specificity and sensitivity together with area under the curve of 1 in the three studied research groups. Multiple regression analysis ascertain the correlation between both antibodies as dependent variables and liver enzymes as independent variables with ALT being the most affected enzyme with CMV seropositivity. This study strongly ascertains that CMV is indeed capable of initiating and accelerating liver dysfunction especially in young people. Serological screening of individuals who are asymptomatic based on a detection of CMV-IgG and IgM might help in early diagnosis and intervention to avoid liver disorder as cirrhosis and other complications related to CMV infection, which are associated with ALT, AST and GGT elevation.

**Keywords:** Cytomegalovirus, alanine transaminase(ALT), aspartate transaminase(AST), alkaline phosphatase(ALP),  $\gamma$ -glutamyltranspeptidase(GGT), liver function.

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Human cytomegalovirus (CMV) as a member of the herpes virus family is one of the

most common infecting viruses in humans. The prevalence of congenital CMV infection varies from 0.2% to 2% (). In developing countries, the reported prevalence of congenital CMV infection varies dramatically within and between populations, with some recorded prevalence ranged from 6.1%

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\* To whom all correspondence should be addressed.  
E-mail: randa792006@gmail.com

to 14.0%<sup>2</sup>. Cytomegalovirus is human-to-human transmissible through close bodily contact, coughs and sneezes<sup>3</sup>. Because CMV infection may occur during delivery, through infected breast milk or by blood transfusion-perinatal transmission are much more prevalent than other congenital infections<sup>4</sup>.

Cytomegalovirus (CMV) remains an important etiological factor for morbidity and mortality of many organ transplant recipients, patients who receive chemotherapy or high-dose corticosteroids or persons infected with human immunodeficiency virus type 1 (HIV-1) (5). It is also a major cause of infantile hepatitis<sup>6</sup>. Based on the World Health Organization (WHO) records, about 40% of all adults worldwide are infected in 2011, indicated by the presence of IgG and IgM in the general population<sup>7</sup>.

Presence of both IgM and IgG in the same sample necessitates avidity testing which helps in differentiating between a recent and non-recent primary infection<sup>8</sup>. Clinically concomitant with the remarkable increase of these antibodies, there is a mild-moderate increase of transaminases, markers of liver function and lymphomonocytosis. There is study by Vujacich, *et al.*, 2006<sup>9</sup> reported a 6 and 3.5 fold increase in alanine transaminase (ALT) and aspartate transaminase (AST) respectively in patients with CMV infection. Here in this study we investigated CMV seroprevalence and liver enzyme profiles in different age groups of Saudi Arabia patients to find the relationship between CMV infection and risk factors for liver dysfunction.

## Subjects and Methods

### Study Population and Specimens

The focus of our study was tested 455 serum samples of patients with elevation of liver profiles (ALT, AST, ALP and GGT) and controls categorized in different age groups were collected from different general hospitals and polyclinics in KSA from March 2014 to June 2016 in different ages and gender. Serum samples which were tested for CMV seropositive (by detection of CMV- IgG and IgM and non- A to G hepatitis virus (non HAV, HBV, HCV, HDV, HEV and HGV). where molecular technical for Extraction of virus nucleic acid, PCR and real-time PCR were performed according to methods described by Lin and Floros, 2000 (10) for selected the samples non- A to G hepatitis virus. Whole blood samples

were collected and centrifuged at 2,000 g for 15 min. Sera were taken and stored at  $-20^{\circ}\text{C}$ . The samples were coded by date of collection, sample number, gender and age. The IRB in the Faculty of Medicine at KSU (Riyadh, KSA) approved the study. Informed consent for the collection of specimens was obtained from all cases to the collection of specimens.

### Serology tests

The CMV IgG and IgM were screened in patients' sera by using a commercially available capture enzyme-linked immunosorbent assay (ELISA) (CMV IgM and CMV IgG, Dia.Pro; Diagnostic BioprobesSrl, Italy). Samples with concentrations  $\geq 1.10$  IU/ml (WHO) were considered to be positive for anti-CMV IgG antibody and samples with concentrations  $\geq 0.9$  IU/ml were interpreted as negative, while samples with concentrations 0.91 – 1.09 IU/ml were considered equivocal. For CMV IgM, samples were considered positive when the ratio of the sample optical density at 450 nm to the cutoff value (signal to cutoff) was  $>1.1$ ; equivocal, 0.9 – 1.0; and negative,  $\leq 0.8$ . ELISA assay results were analyzed in all samples included in the study.

### Statistical analysis

SPSS software was used for statistical analysis. Results were expressed as mean  $\pm$  SD and all statistical comparisons were made by means of independent t-test with  $p < 0.05$  considered as significant. Pearson's correlations between measured parameters were also presented. Receiver Operating Characteristic (ROC) analysis was performed as a comprehensive way to assess the accuracy of the studied markers as previously described (11). The area under the curve (AUC) was used provides a useful metric to compare IgG and IgM as two CMV seropositivity markers. Whereas an AUC value close to 1 indicates an excellent diagnostic and predictive marker, a curve that lies close to the diagonal (AUC = 0.5) has no diagnostic utility. AUC close to 1 is always accompanied by satisfactory values of specificity and sensitivity of the biomarker.

Multiple regression analysis was also used to find the correlation between the IgG, IgM and different liver enzymes. In this analysis  $R^2$  described the proportion or percentage of variance in the dependent variable (IgG and IgM) explained by the variance in the independent variables

together which sometimes called the predictor variables (AST, ALT, ALP and GGT). An R2 of 1.00 indicates that 100% of the variation in the dependent variable is explained by the independent variables. Conversely, an R2 of 0.0 indicates the absence of variation in the dependent variable due to the independent variables. The  $\beta$  coefficients

values showed the direction either positive or negative and the contribution of the independent variable relative to the other independent variables in explaining the variation of the dependent variable. R2 and ( $\beta$ ) coefficient provide most of what we need to interpret our multiple regression data.

**Table 1.** Comparisons of ALT, AST, ALP, GGT, IgG and IgM in the three studied age groups of CMV seropositive patients compared to healthy control participants

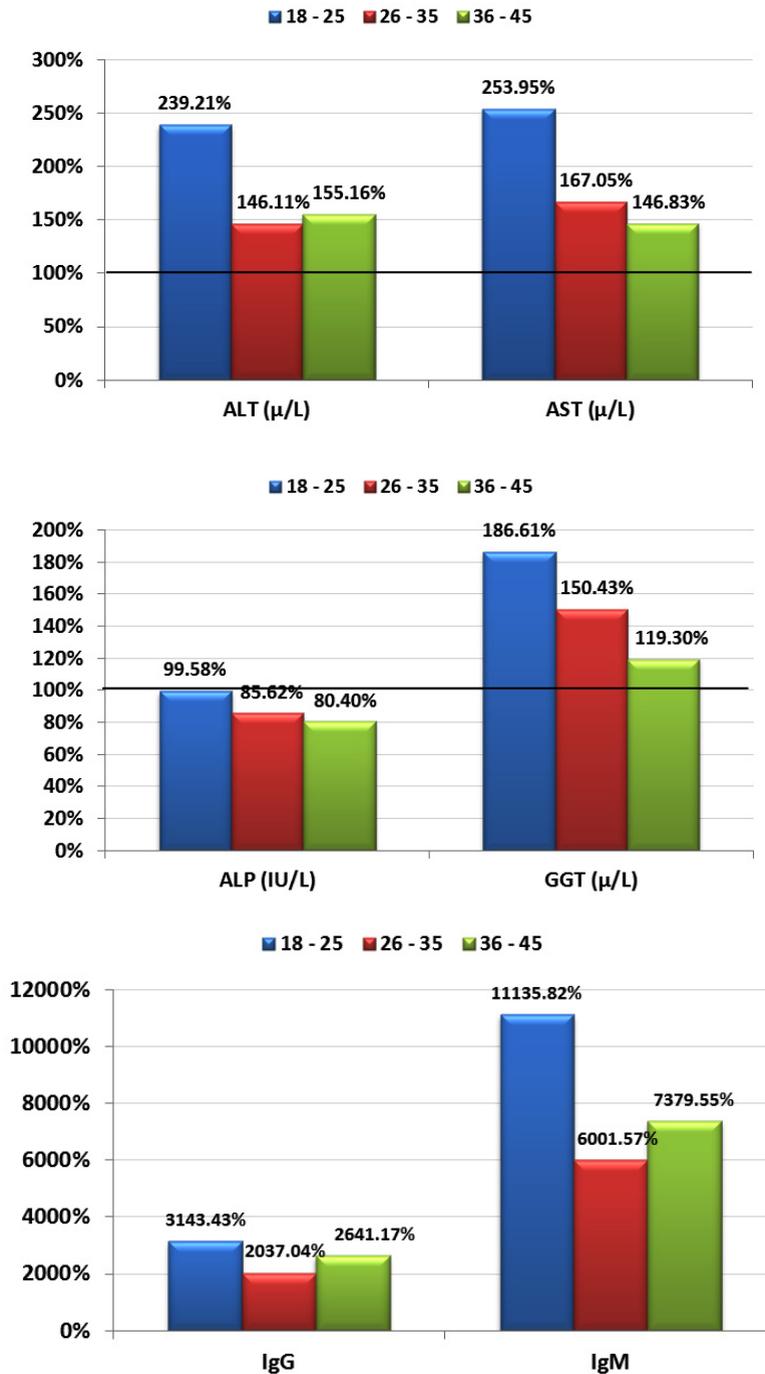
Parameter	Age Groups	Group	N	Min.	Max.	Mean $\pm$ S.D.	Percent Change	P value
ALT ( $\mu$ /L)	18 - 25	Control	15	17.06	35.09	25.62 $\pm$ 5.37	100.00	0.001
		Patients	150	55.78	95.47	61.29 $\pm$ 7.35	239.21	
	26 - 35	Control	10	33.01	55.80	40.94 $\pm$ 10.14	100.00	0.033
		Patients	165	56.20	76.22	59.81 $\pm$ 3.71	146.11	
	36 - 45	Control	15	35.29	45.25	40.77 $\pm$ 3.33	100.00	0.001
		Patients	140	56.33	76.22	63.25 $\pm$ 5.35	155.16	
AST ( $\mu$ /L)	18 - 25	Control	15	12.56	22.23	17.09 $\pm$ 2.70	100.00	0.001
		Patients	150	35.08	62.71	43.39 $\pm$ 6.29	253.95	
	26 - 35	Control	10	22.72	27.72	25.24 $\pm$ 2.05	100.00	0.001
		Patients	165	35.08	62.01	42.15 $\pm$ 5.58	167.05	
	36 - 45	Control	15	26.42	32.90	29.39 $\pm$ 1.92	100.00	0.001
		Patients	140	35.23	58.50	43.15 $\pm$ 5.11	146.83	
ALP (IU/L)	18 - 25	Control	15	49.31	148.20	84.89 $\pm$ 31.69	100.00	0.971
		Patients	150	45.34	148.20	84.54 $\pm$ 32.61	99.58	
	26 - 35	Control	10	78.45	120.00	96.50 $\pm$ 17.86	100.00	0.397
		Patients	165	45.02	148.20	82.63 $\pm$ 31.58	85.62	
	36 - 45	Control	15	67.23	137.30	94.97 $\pm$ 21.87	100.00	0.133
		Patients	140	45.02	147.30	76.35 $\pm$ 30.98	80.40	
GGT ( $\mu$ /L)	18 - 25	Control	15	12.02	58.46	31.80 $\pm$ 12.17	100.00	0.001
		Patients	150	34.23	88.32	59.34 $\pm$ 14.73	186.61	
	26 - 35	Control	10	27.37	48.82	39.39 $\pm$ 9.07	100.00	0.008
		Patients	165	34.23	88.32	59.26 $\pm$ 13.68	150.43	
	36 - 45	Control	15	40.34	57.37	50.71 $\pm$ 6.53	100.00	0.010
		Patients	140	40.34	72.01	60.50 $\pm$ 11.90	119.30	
IgG	18 - 25	Control	15	0.15	0.62	0.38 $\pm$ 0.16	100.00	0.001
		Patients	150	3.81	35.05	11.85 $\pm$ 6.38	3143.43	
	26 - 35	Control	10	0.24	0.72	0.48 $\pm$ 0.21	100.00	0.001
		Patients	165	4.05	19.06	9.78 $\pm$ 4.08	2037.04	
	36 - 45	Control	15	0.14	0.82	0.40 $\pm$ 0.21	100.00	0.001
		Patients	140	5.22	19.06	10.60 $\pm$ 5.23	2641.17	
IgM	18 - 25	Control	15	0.11	0.37	0.24 $\pm$ 0.09	100.00	0.001
		Patients	150	12.34	90.29	26.89 $\pm$ 16.95	11135.82	
	26 - 35	Control	10	0.10	0.52	0.34 $\pm$ 0.18	100.00	0.001
		Patients	165	12.04	33.56	20.26 $\pm$ 4.64	6001.57	
	36 - 45	Control	15	0.11	0.42	0.27 $\pm$ 0.09	100.00	0.001
		Patients	140	12.34	34.74	20.21 $\pm$ 4.22	7379.55	

•Table 1 describes the independent *t*-test between control and patients categorized in 3 different age groups (18 - 25, 26 - 35 and 36 - 45) for all parameters

**RESULTS**

Table 1 and figure 1 demonstrate the high significant elevations of AST, ALT and GGT

together with the non-significant change in ALP. It can be easily noticed that both transaminases (ALT and AST) together with GGT were markedly higher in the three studied age groups with the first



**Fig. 1.** (a,b,c) Percentage change of IgG and IgM all parameters in each age group

age group (18-25 years) recording the much higher percentage increase for of ALT (139.21%) and for AST (153.95%) compared to a much lower increase in the other two studied age groups.

While the data in and Table 2 and figure 2 show the significant correlations between both IgG and IgM as measures of CMV seropositivity. In addition, significant correlations were also recorded between these two antibodies and AST, ALT, and GGT as measures of liver injury risk (e.g. liver cirrhosis).

Table 3 and figure 3 represent the receiver operating characteristics (ROC) analysis of IgG and IgM in male and females CMV- seropositive patients. An area under the curve equal to 1 together with 100% sensitivity and specificity were recorded for both antibodies in the three age groups of CMV patients.

The results in Table 4 and 5 show the stepwise multiple regression analysis using IgG and IgM as dependent variable respectively and AST, ALT, ALP and GGT as independent variables.

Adjusted R<sup>2</sup> and  $\beta$  coefficients of the different variables are listed.

## DISCUSSION

CMV infection may have important health consequences. Many studies propose that infection with CM reduce the performance of the immune system to respond to further antigenic challenge and increase the risk of developing liver dysfunction<sup>12, 13, 14</sup>. This work aims to extend these findings by investigating the effect of age on prevalence of CMV as non hepatotropic agent infection in a large number of samples that included different range of ages. The provision of information on hygiene may be an effective and inexpensive method for preventing CMV infection and control its role on liver dysfunction in future.

Patients with liver enzyme deviations are usually divided into two categories either alkaline phosphatase (ALK) or transaminases (ALT and AST) elevation. In case of alkaline phosphatase,

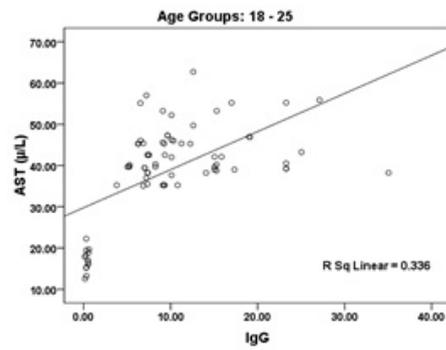
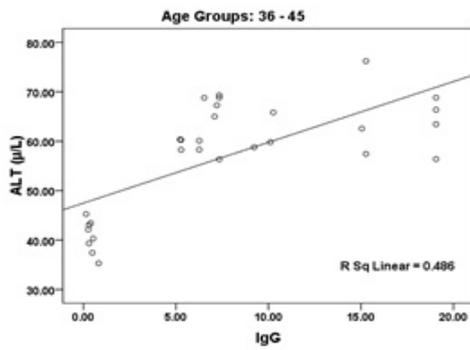
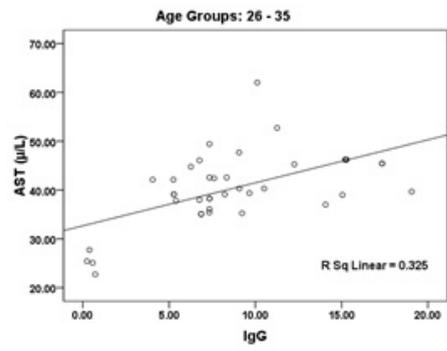
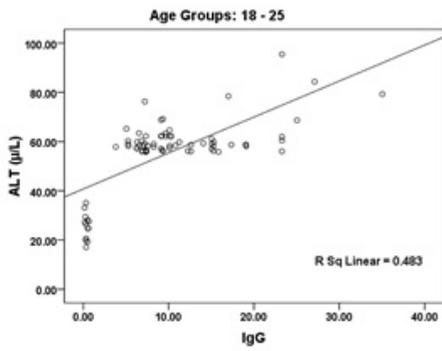
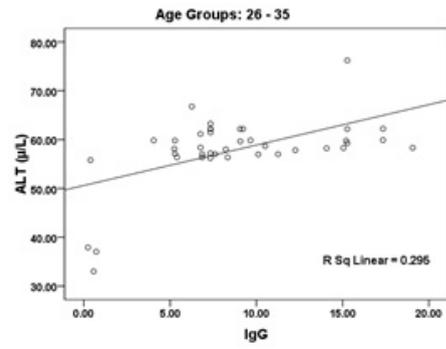
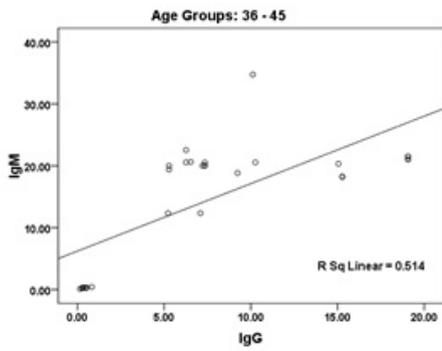
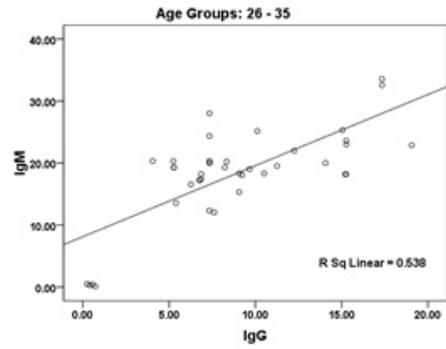
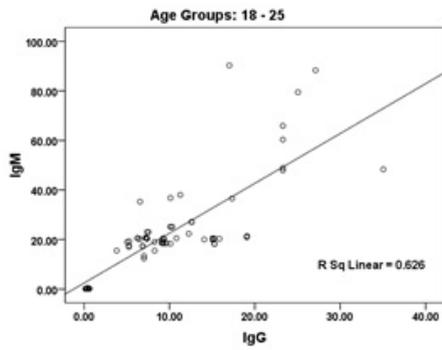
**Table 2.** Pearson's correlations between the measured parameters

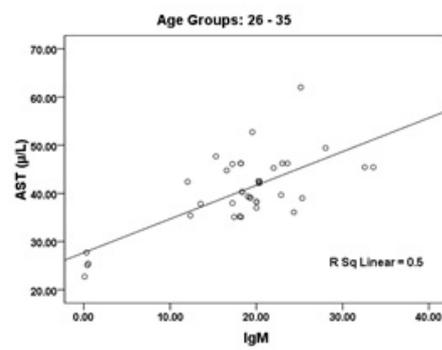
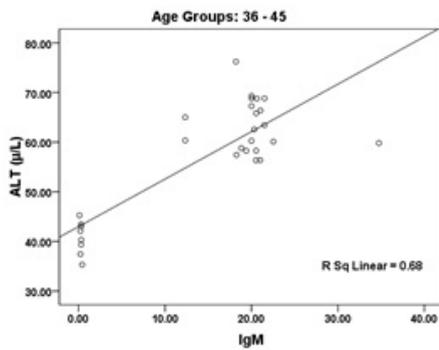
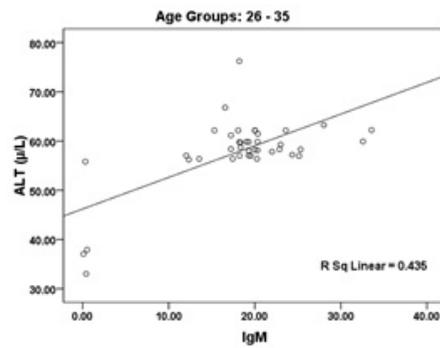
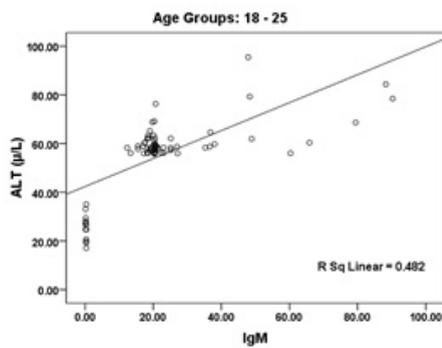
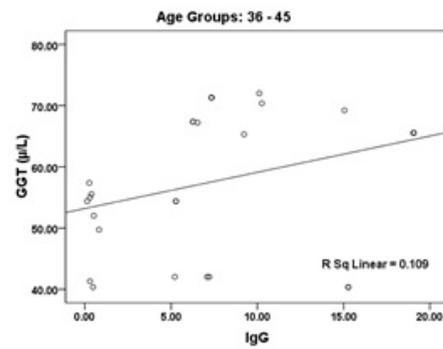
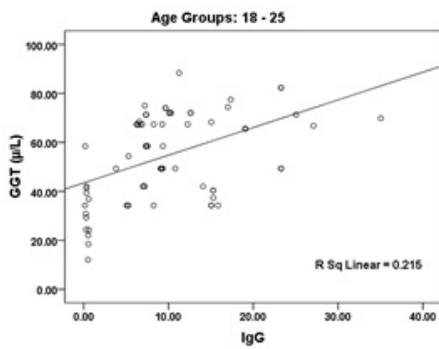
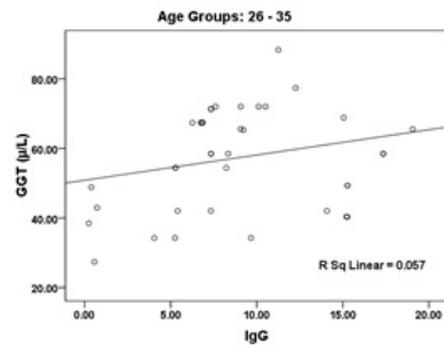
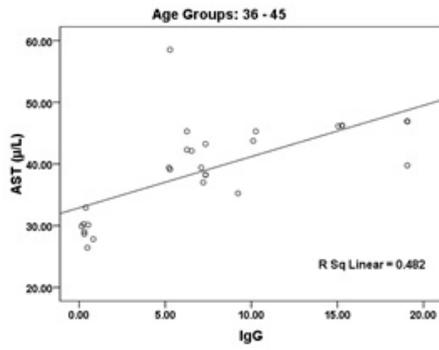
Parameters	Age Groups	R (Person Correlation)	Sig.	
IgG ~ IgM	18 - 25	0.791**	0.001	P <sup>a</sup>
	26 - 35	0.734**	0.001	P <sup>a</sup>
	36 - 45	0.717**	0.001	P <sup>a</sup>
IgG ~ ALT ( $\mu$ /L)	18 - 25	0.695**	0.001	P <sup>a</sup>
	26 - 35	0.544**	0.001	P <sup>a</sup>
	36 - 45	0.697**	0.001	P <sup>a</sup>
IgG ~ AST ( $\mu$ /L)	18 - 25	0.580**	0.001	P <sup>a</sup>
	26 - 35	0.570**	0.001	P <sup>a</sup>
	36 - 45	0.694**	0.001	P <sup>a</sup>
IgG ~ GGT ( $\mu$ /L)	18 - 25	0.464**	0.001	P <sup>a</sup>
	26 - 35	0.240	0.137	P <sup>a</sup>
	36 - 45	0.331	0.080	P <sup>a</sup>
IgM ~ ALT ( $\mu$ /L)	18 - 25	0.695**	0.001	P <sup>a</sup>
	26 - 35	0.659**	0.001	P <sup>a</sup>
	36 - 45	0.825**	0.001	P <sup>a</sup>
IgM ~ AST ( $\mu$ /L)	18 - 25	0.586**	0.001	P <sup>a</sup>
	26 - 35	0.707**	0.001	P <sup>a</sup>
	36 - 45	0.800**	0.001	P <sup>a</sup>
IgM ~ GGT ( $\mu$ /L)	18 - 25	0.532**	0.001	P <sup>a</sup>
	26 - 35	0.383*	0.015	P <sup>a</sup>
	36 - 45	0.548**	0.002	P <sup>a</sup>

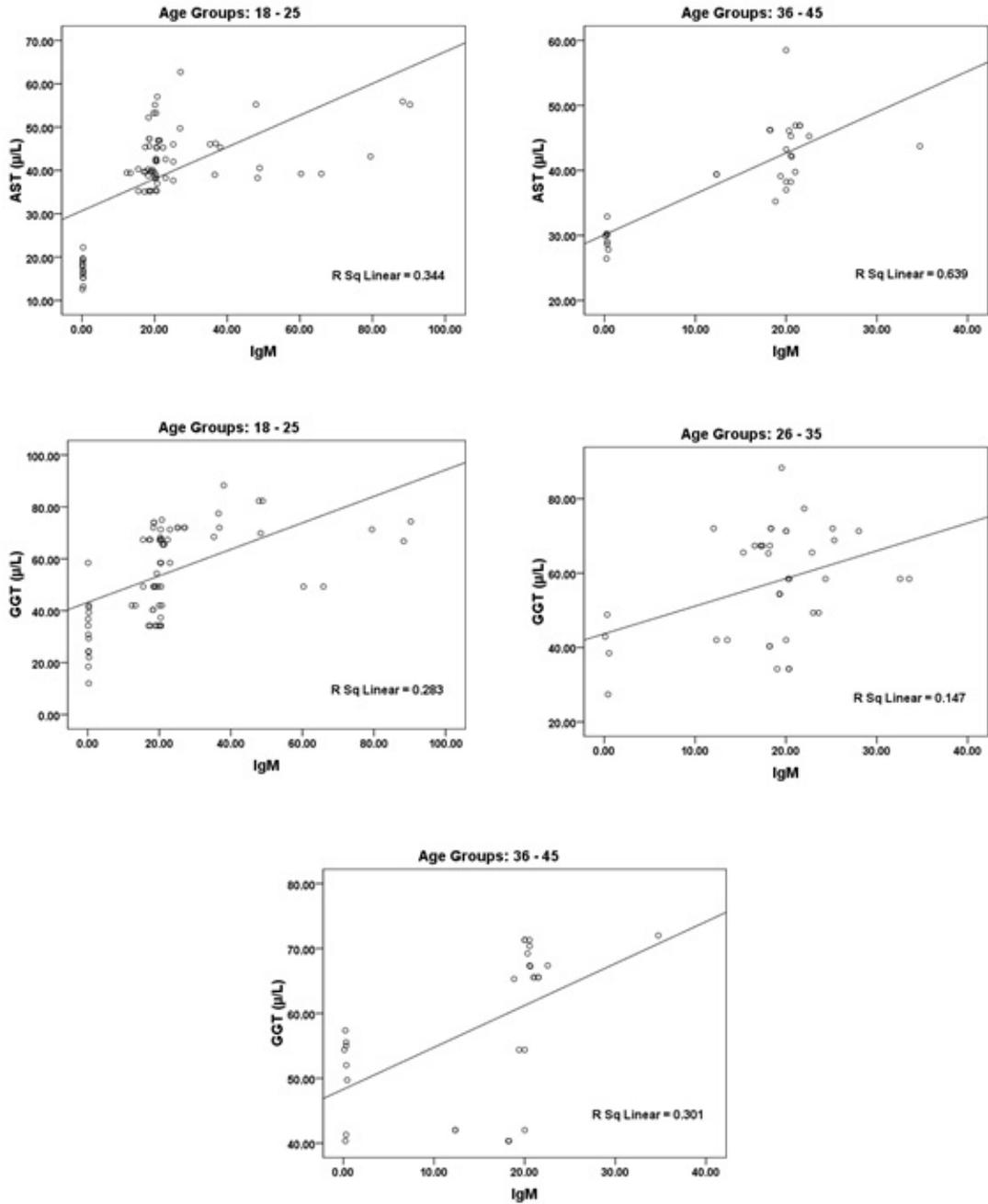
\*Correlation is significant at the 0.05 level.

\*\*Correlation is significant at the 0.01 level.

<sup>a</sup>Positive Correlation.







**Fig. 2.** Correlation between all parameters according in each age group (18 - 25, 26 - 35 and 36 - 45) with best fit line curve (positive correlation)

patients generally have either cholestasis disease or infiltrative disease. Transaminases elevation is predominant and can be a marker of hepatocellular dysfunction due to viral hepatitis, autoimmune

hepatitis or liver toxins.<sup>3</sup>-Glutamyltranspeptidase (GGT) levels tend to parallel alkaline phosphatase elevations that originate from the liver<sup>15,16</sup>.

The data in Table 1 demonstrates the

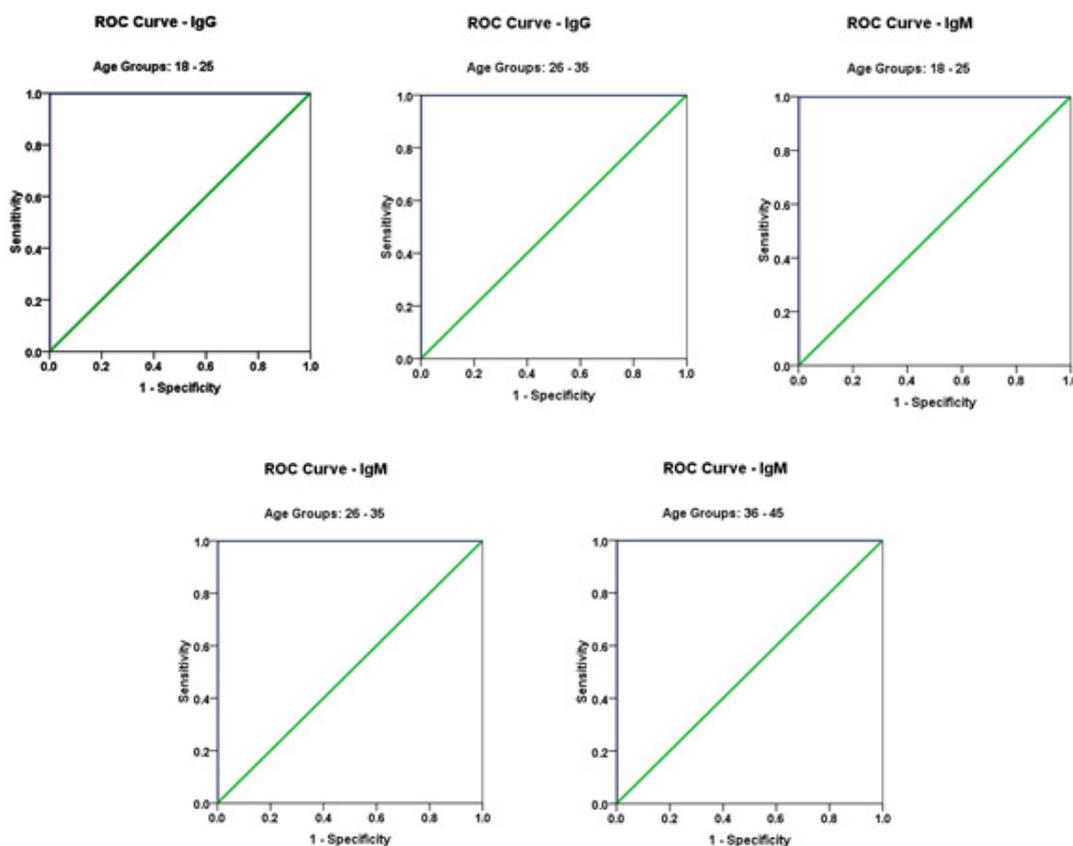
high significant increase of both AST and ALT together with the non-significant elevation of ALP. This can ascertain the possibility to develop liver dysfunction in CMV patients. It can be easily noticed that age group 1<sup>18-25</sup> was at higher risk to develop liver dysfunction compared to the other 2 elder groups (26-35 & 36-45) which recorded

much lower increase in both transaminases. Effect of CMV infection in inducing both transaminases as markers of liver dysfunction was confirmed through the elevation of IgG and IgM. as antibodies against CMV infection.

Elevated transaminases were significantly associated with both CMV seropositivity (i.e CMV

**Table 3.** ROC-Curve of IgG and IgM olacording the Age groups (18 – 25 , 26 – 35 and 36 – 45 ) in Patients group

	Group	Area under the curve	Cut-off value	Sensitivity %	Specificity %
IgG	18 - 25	1.000	2.215	100.0 %	100.0 %
	26 - 35	1.000	2.385	100.0 %	100.0 %
	36 - 45	1.000	3.020	100.0 %	100.0 %
IgM	18 - 25	1.000	6.355	100.0 %	100.0 %
	26 – 35	1.000	6.280	100.0 %	100.0 %
	36 – 45	1.000	6.380	100.0 %	100.0 %



**Fig. 3.** ROC Curve of IgG according the age (18 - 25 and 26 – 35) and IgM according age group of patients 18 - 25 , 26-35 and 36 – 45

**Table 4.** Multiple Regression using stepwise method for IgG as a dependent variable

Age Groups	Predictor Variable	Beta	P value	Adjusted R <sup>2</sup>	Model	
					F value	P value
18 - 25	ALT (μ/L)	0.331	0.001	0.476	69.196	0.001
26 - 35	AST (μ/L)	0.369	0.001	0.307	18.270	0.001
36 - 45	ALT (μ/L)	0.395	0.001	0.467	25.520	0.001

**Table 5.** Multiple Regression using stepwise method for IgM as a dependent variable

Age Groups	Predictor Variable	Beta	P value	Adjusted R <sup>2</sup>	Model	
					F value	P value
18 - 25	ALT (μ/L)	0.840	0.001	0.475	68.953	0.001
26 - 35	AST (μ/L)	0.715	0.001	0.487	37.967	0.001
	AST (μ/L)	0.358	0.017	0.548	24.675	0.001
36 - 45	ALT (μ/L)	0.711	0.001	0.669	57.468	0.001
	GGT (μ/L)	0.258	0.006			
	ALT (μ/L)	0.402	0.003	0.784	34.896	0.001
	GGT (μ/L)	0.434	0.024			
	AST (μ/L)	0.231	0.008			

patients compared to control) and high CMV antibody levels (age group 1 compared to age groups 2 and 3). Comparisons of the 3 specific age groups revealed that this association was detectable early in life (18-25 years of age).

This is in good agreement with the previous study of Lopo *et al*, 2011<sup>13</sup> in which they study the prevalence of CMV infection in 8 age groups of Portuguese population range from 2 to 65. They recorded that while the antibody prevalence in children at school age (age groups 5-9 years and 10-14 years) was more or less similar to that at pre-school age, it was increased to reach 71.3% in the age group between 15 and 19 years, which corresponds to a greater sexual exposure. In addition to non- sexual contact<sup>11,12, 15</sup>.

Studies with similar age groups conducted in other countries, such as the United States, Japan, France, England, Poland and Russia, describe seroprevalences ranging between 51.5% and 78.0% (17, 18, 19) which is still lower than the 136% increase recorded for 18-25 years age group of the present study. As it is well known that sexual transmission is a likely risk factor for exposure to CMV (20) so the remarkable high seropositivity

in the 18-25 age group compared to the other 2 groups can be attributed to the more frequent sex intercourse, as a significant predictor of CMV infection. This much higher CMV seropositivity can be connected with the ethnic/ socioeconomic status of Saudi population. This can be supported by the study of Ghazi *et al*. 2002, which recorded a prevalence of 92.1% CMV total IgG antibodies in pregnant Saudi women<sup>20</sup>.

In Table 2 and figure 2 the data showing Pearson's correlations between ALT, AST, ALP and GGT in one hand and IgG and IgM in the other hand. It can be easily noticed that both antibodies were positively correlated with high significant difference. This can suggest the importance of both as markers of CMV seropositivity. In addition both antibodies were independently correlated with the four measured liver enzymes which can ascertain the concomitant liver dysfunction associated with CMV infection. This can find a support in the recent work of<sup>21, 22</sup> which prove that CMV infection usually induces autoimmune hepatitis and primary biliary cirrhosis. This can explain the high significant positive correlation between CMV antibodies and AST, ALT and ALP in the three age groups tested.

The receiver operating characteristics (ROC) curve as a fundamental tool for biomarkers evaluation was performed, using the same computer program. In a ROC curve the true positive rate (sensitivity) is plotted as a function of the false positive rate (100-specificity) for different cut-off points of a parameter. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold. The area under the ROC curve is a measure of how well a parameter can distinguish between CMV seropositive and control subjects. The area under the curve (AUC) provides a useful measure to compare different biomarkers. Whereas an AUC value close to 1 indicates an excellent diagnostic and predictive marker, a curve that lies close to the diagonal (AUC = 0.5) has no diagnostic utility. AUC close to 1 is always accompanied by satisfactory values of specificity and sensitivity of the biomarker. From 0.9 to 1 considered as excellent, 0.8 to 0.9 as good, 0.7-0.8 as fair, 0.6-0.7 as poor and 0.5-0.6 as fail. Based on this information, both antibodies can be used as excellent predictive markers for CMV infection in all three tested age groups.

1. In the present study, the general purpose of multiple regressions is to learn more about the relationship between several independent or predictor variables (AST, ALT, ALP and GGT), and a dependent or criterion variable (IgG or IgM) through the recorded R<sup>2</sup> and  $\beta$  coefficient values. In this analysis R<sup>2</sup> describes the proportion or percentage of variance in the dependent variable explained by the variance in the independent variables together which sometimes called the predictor variables. An R<sup>2</sup> of 1.00 indicates that 100% of the variation in the dependent variable is explained by the independent variables. Conversely, an R<sup>2</sup> of 0.0 indicates the absence of variation in the dependent variable due to the independent variables. The  $\beta$  coefficients values show the direction either positive or negative and the contribution of the independent variable relative to the other independent variables in explaining the variation of the dependent variable. R<sup>2</sup> and ( $\beta$ ) coefficient provide most of what we need to interpret our multiple regression data. Table 4 and 5 demonstrate the linear regression analysis between the measured parameters using IgG and IgM as dependent variables respectively. It can be

easily noticed that both IgG and IgM show low R<sup>2</sup> values. This suggests was expected as CMV as viral infectious disease never induced in case of liver dysfunction (22). On the other hand,  $\beta$  coefficients values for AST, ALT and GGT as independent variables point out that ALT is the most important enzyme related to CMV seropositivity measured by high IgG and IgM. ALT recorded  $\beta$  coefficients values of 0.840 and 0.711 in 18-25 and 26-35 age groups respectively.

In Conclusion, our results indicate a high significant association between CMV seropositivity and increased levels of AST, ALT, and GGT, as markers of liver dysfunction that may lead to liver cirrhosis in patients without hepatotropic viruses such as HCV and HBV. Based on other studies showing that CMV seroprevalence in patients with hepatocellular carcinoma (HCC) is significantly higher than in patients without HCC and is positively correlated with liver cirrhosis<sup>23, 24</sup>. Therefore, elimination of CMV infection via the development and administration of treatments or vaccines may reduce HCC mortality rates<sup>24</sup>. Therefore, elimination of CMV infection which is a potentially feasible and important task for preventing diseases linked to CMV infection<sup>25</sup>. Future studies will be needed to further define the role of CMV in liver disorder.

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