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ORIGINAL ARTICLE

THE ASSOCIATION BETWEEN HUMAN CYTOMEGALOVIRUS INFECTION AND SERUM MIDKINE LEVELS WITH PROGRESSION OF GASTRIC CARCINOMA

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ABSTRACT

Gastric cancer (GC) is the fifth most frequently diagnosed malignancy and the third most common cause of cancer-related fatalities worldwide. One of the human viruses that has received the most research is the cytomegalovirus (CMV), which may occasionally induce gastritis, especially in people with an impaired immune system. It is uncertain whether CMV gastritis aids in the growth of stomach cancer. This study was performed to assess the relationship between CMV DNA and serum midkine (S-MK) levels in patients with stomach cancer of the adenocarcinoma subtype. A total of 120 serum samples were collected, including 45 (37.5%) samples of each GC and gastric precancerous lesions, and 30 (25%) of control samples. DNA was extracted from all the serum samples. Real-time PCR was performed to detect CMV DNA, and midkine levels were measured by ELISA. Thirty-five (29.2%) samples of GC, nine (7.5%) samples of gastric precancerous lesions, and five (4.2%) of control samples were positive for CMV DNA. Interestingly, there was a gradual increase in S-MK levels in GC patients (516.25 ± 150.86 pg/mL), followed by gastric lesions (189.72 ± 76.63 pg/mL) and then healthy controls (86.96 ± 83.95 pg/mL) (p= 0.001). Finally, it was determined that human CMV infection may be linked to the development or progression of GC and may correlate with serum midkine levels, which have excellent diagnostic value and can be used to track GC prognosis and aid physicians.

KEY WORDS: CMV; gastric cancer; gastric adenocarcinoma; gastric lesions; S-MK.

INTRODUCTION

One of the most prevalent cancers in the world is gastric cancer, sometimes referred to as stomach cancer. It starts in the cells lining the stomach and spreads to other parts of the body. Gastric cancer is caused by several variables, such as genetics, lifestyle decisions, and infections (Ajani et al., 2022). A common virus in

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the herpesvirus family is the cytomegalovirus (CMV), which affects a significant proportion of the world's population and is typically latent and asymptomatic. CMV, however, can have serious side effects and result in illnesses in some people, particularly those with a compromised immune system (Parija, 2023).

Scientists and medical experts have been interested in and researching the association between CMV infection and stomach cancer. Some research suggests that chronic CMV infection might raise the risk of stomach cancer. It is important to remember that this relationship is complex and not yet fully understood. The discovery of CMV DNA in stomach cancer tissues in some investigations raises the possibility that the virus contributes to the development or spread of the disease. However, the precise mechanism by which CMV may aid in the emergence of stomach cancer is yet unknown (Lv et al., 2020). The propensity of CMV to cause persistent inflammation and immunosuppression is one explanation for the association between the virus and stomach cancer. Chronic inflammation can damage healthy cells' DNA and promote tumor development. Furthermore, immunosuppression may hamper the immune system's defenses against cancer cells, allowing them to grow unchecked (Varani & Landini, 2011). Several biological activities, including cell proliferation, survival, migration, and inflammation, depend on the protein called midkine. In healthy tissues, it is typically expressed at low levels, but in many illnesses, including cancer, it is elevated. Researchers have looked into the possible utility of midkine as a biomarker for several malignancies, including gastric cancer, over the years. Midkine serum levels have drawn interest as a potential diagnostic and prognostic marker for gastric cancer. Numerous studies have looked into the relationship between midkine levels and the occurrence, progression, and overall prognosis of gastric cancer (Ji et al., 2020). Thus, this study was conducted to assess the association of CMV DNA and serum midkine (S-MK) levels in patients with gastric cancer.

MATERIAL AND METHODS

Study population and specimens

This cross-sectional study of serum samples was conducted on 120 patients admitted between January and July 2023 at the Gastroenterology and Hepatology Teaching Hospital and Ghazi Al-Hariri Hospital for Surgical Specialties, both part of Baghdad Medical City. The study included 45 patients (24 males and 21 females), aged 25 to 73 years, diagnosed with gastric adenocarcinoma by postoperative pathology. Additionally, 45 patients (24 males and 21 females), aged 25 to 70 years, diagnosed with gastric precancerous lesions, were treated surgically and are currently attending the aforementioned hospitals as part of this study. Additionally, a control group of 30 serum samples from healthy blood donors was selected based on standard

blood testing (13 males and 17 females, with a mean age of 45.11 years and a range of 25 to 72 years). The sample size was determined based on feasibility within the study period and on prior studies evaluating CMV and serum biomarkers in gastric cancer. No formal power calculation was conducted. All available demographic data and information were obtained from the patients' pathologic and medical reports: age, sex, clinical presentation, tumor location, histology, grade, and stage, depth of wall invasion, lymph node metastasis, and vascular invasion. Based on Lauren's classification, GC histological types were divided into categories. According to the eighth edition of the AJCC/UICC Classification for Carcinoma of the Stomach, GC's TNM (Pathologic Tumor-Node-Metastasis) staging was performed. The inclusion criteria: all untreated GC patients. The exclusion criteria: patients undergoing chemotherapy and radiation therapy.

Ethical approval

The Middle Technical University (MTU) College of Health and Medical Techniques' Ethical Committees approved the study under reference number 9185, 02/05/2022. Because all specimens were coded and patient names were replaced with codes, the analysis used anonymized clinical data collected after each patient consented to treatment in writing.

Genomic DNA extraction for CMV detection

DNA was extracted and purified from serum using the gSYNCTM DNA extraction kit's fast technique (Geneaid Biotech Ltd. GS100®). Proteinase K and a chaotropic salt were used in the kit to lyse cells and break down protein, allowing DNA to bind to the glass fiber matrix of the spin column. The purified genomic DNA was eluted with water after contaminants were eliminated with a wash buffer. Genomic DNA integrity was assessed using gel electrophoresis. The purity and concentration of the extracted DNA were evaluated using a NanoDrop. 5 µl of the extracted DNA was placed in the instrument sample cell for analysis. An extract with a purity of (1.8 - 2) at absorption wavelength 260/280 was accepted; otherwise, DNA extraction from the sample would be carried out. To detect CMV infection, a PCR was performed using the obtained DNA.

Real-time PCR for CMV DNA detection

The TaqMan® technology-based Cytomegalovirus (CMV) TaqMan PCR Kit from Norgen Biotek Inc. (Canada; # TM36350®) was used to identify CMV-specific DNA in a real-time PCR. (Ready-to-Use) was used; it contains a PCR control to check for PCR inhibition, a Master Mix for target and PCR

control detection, primer and probe mix, as well as a positive control and a negative control (nuclease-free water) to ensure the integrity of the kit reagents. All TaqMan PCR components were thoroughly melted at room temperature, mixed by gentle vortexing, and briefly centrifuged. For accurate interpretation, two reactions — one containing a CMV-positive control and the other without a template — were included in each TaqMan PCR run.

The TaqMan PCR test, the negative control, and the positive control were all created without any contamination using the PCR CMV test. To minimize the risk of contamination, the components were added to the PCR tubes in the following order: (1) nuclease-free water; (2) MDx TaqMan 2× PCR Master Mix; (3) primer-probe mix; and (4) sample DNA or positive control. Approximately 3 μL of sample DNA was recommended for use. However, a volume of sample DNA ranging from 1 to 5 μL may be employed as a template. The final volume of the PCR reaction was adjusted to 20 μL.

The Real-time PCR (CFX96: BioRad, USA ®) thermocycler was used in the last step of the thermal protocol, and it was set to have the thermal cycling conditions as follows: initial denaturation at 95 °C for 3 min (1 cycle), followed by (40 cycles) of denaturation at 95 °C for 15 seconds and 60 °C for annealing and extension. The amplification curve was provided for each sample, with the (X) axis representing the Ct (threshold cycle) and the (Y) axis showing CMV DNA or the internal control, expressed as Relative Fluorescence Units (RFU), detected on the Hex/Orange channel. The instrument software automatically determined the baseline cycles and threshold values. Qualitative results were displayed in report mode. To prevent false-negative results, internal control was analyzed in samples with undetectable signals. Samples that crossed the threshold in the FAM (green) channel were interpreted as positive, whereas those without amplification (NO CT) were considered negative.

Serum midkine (S-MK) measurement

Blood samples (5 mL) were collected by venipuncture and immediately centrifuged at 3,000 × g for 10 min to obtain serum that was stored at -80 ºC until analysis. Quantitative measurement of human midkine (MK) concentrations in serum and plasma was carried out using a commercial ELISA kit (CUSABIO Technology LLC. ELISA, USA ®), following the manufacturer's protocol.

Statistical analysis

The mean and standard deviation of quantitative data with a normal distribution were displayed, while the median (min-max) and frequency tables, along with an analysis of variance (ANOVA) test, were used to characterize other data. All estimated *p values* were two-sided, and a significance level

of $p<0.05$ was typically accepted. Receiver operating characteristic (ROC) curves were created to assess a parameter's ability to discriminate. The best cut-off values were selected based on the areas under the ROC curves (AUC). Sensitivity, specificity, diagnostic accuracy, PPV, and NPV for differential diagnosis were then evaluated accordingly. The IBM SPSS Statistics software version 25.0 was used for all statistical studies (Schober & Vetter, 2019; Hajian-Tilaki, 2013®). All 120 participants had complete datasets for CMV-DNA and S-MK levels. No imputation methods were required.

RESULTS

Study population, tumor samples, and clinical data

In this study, a total of 120 subjects were enrolled, with 45 (37.5%) samples of each GC and gastric precancerous lesions, and 30 (25%) of control samples. The CMV-DNA results showed that 49 (40.8%) were positive and 71 (59.2%) were negative, as presented in Table 1. Subjects were 61 males (49.2%) and 59 females (50.8%) with a median age of 48 years (range: 24-77 years) and were classified according to the TNM guidelines as follows: 3 (6.7%) stage I; 9 (20%) stage II; 23 (51.1%) stage III; and 10 (22.2%) stage IV. Patients with stage IV disease showed distant lymph node metastasis in 26 (57.8%). Nearly 32 (71.1%) of the tumors were of the diffuse type according to Lauren's criteria, and the tumors were distributed as follows: in the antrum 12 (26.7%), in the body 16 (35.6%), and the cardia 17 (37.8%). The majority of cases were aggressive: T3 or T4 in 30 (66.7%) and lymph nodular metastasis (N1) in 22 (48.9%), as shown in Figure 1.

Table 1. Variables characteristics of study groups.

Study group	Variables	*n* (%)
	Control	30 (25%)
	Gastric precancerous lesions	45 (37.5%)
	Gastric adenocarcinomas	45 (37.5%)
Sex	Male	61 (49.2%)
	Female	59 (50.8%)
Age categories	< 50 years	63 (52.5%)
	≥ 50 years	57 (47.5%)
*CMV-DNA test	Positive	49 (40.8%)
	Negative	71 (59.2%)
**S-MK levels	High	52 (43.3%)
	Low	68 (56.7%)

*CMV= cytomegalovirus, **S-MK= serum midkine

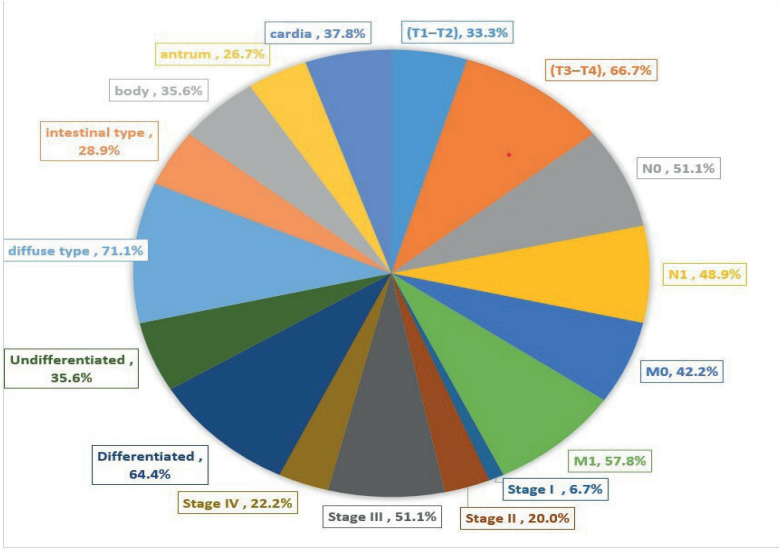

Figure 1. Clinicopathological characteristics of patients with gastric adenocarcinoma. The pie chart illustrates the distribution of tumor staging, nodal involvement, metastasis status, vascular invasion, and histological differentiation. Tumor stage is represented as early (T1–T2, 33.3%) and advanced (T3–T4, 66.7%). Lymph node status shows N0 (51.1%) and N1 (48.9%). Distant metastasis is distributed as M0 (42.2%) and M1 (57.8%). Pathological staging is classified into stages I (6.7%), stages II (20%), III (51.1%), and IV (22.2%). Histological grading indicates differentiated (64.4%) and undifferentiated (35.6%) types. Additional clinicopathological subtypes are shown on the right panel, including diffuse type (71.1%), intestinal type (28.9%), and tumor locations: antrum (26.7%), body (35.6%), and cardia (37.8%).

The ROC analysis for (S-MK) levels

The median serum midkine level in our study was 205 pg/mL, with a range of 24 to 890 pg/mL. The ideal cut-off point was 296 pg/mL, with an area under the receiver operating characteristic curve of 0.99, a *p*-value of <0.001, and a 95% confidence interval of 0.98 to 1.0 (Figure 2). Subjects with serum midkine concentrations ≤ 296 pg/mL were divided into two categories: low 68 (56.7%) and high 52 (43.3%), the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, and accuracy were each 77.5%, 74.7%, 62.2%, 89.1%, 2.73, and 0.30, respectively.

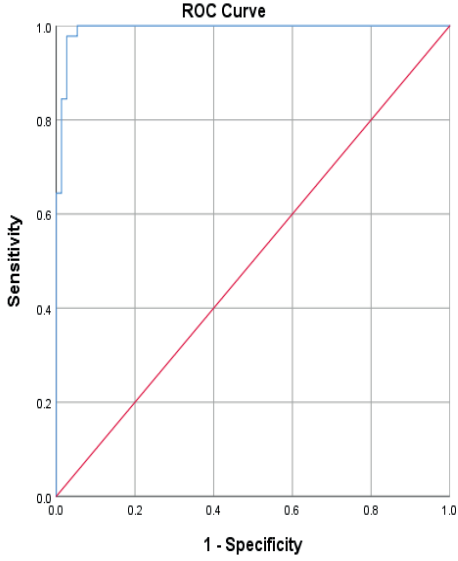

Figure 2. Receiver operating characteristic curve for S-MK in the context of discrimination among study groups.

The S-MK levels in patients with malignant gastric cancer (516.25±150.86 pg/mL) were remarkably higher than those with gastric lesions (189.72±76.63 pg/mL) or healthy controls (86.96±83.95 pg/mL) (Table 2, *p*=0.001).

Table 2. S-MK (pg/mL) levels for study groups.

Study groups	N	Mean ± Std. Deviation	F	df	*p-value*
Control	30	86.96 ± 83.95 pg/mL	159.458	2	
pre-GC	45	189.72±76.63 pg/mL	291.220	1	< 0.001
*GC	45	516.25±150.86 pg/mL	27.697	1	
Total	120	286.48±214.11 pg/mL		119	

*GC= gastric cancer, F: F-value, df: degrees of freedom.

Association between HCMV infection and demographic parameters of the study groups

In this survey, 45 (37.5%) samples of GC and 30 (25%) of control samples were positive for CMV DNA (p= 0.001), as 35 (29.2%) and 5 (4.2%), respectively, in comparison with patients with precancerous gastric lesions, which were 9 (7.5%). The frequency of positive CMV was among the age group < 50 years, 26 (21.7%), compared to ≥ 50 years, 23 (19.2%), (p=0.73). 24 (20%) of male patients with GC tested positive for CMV DNA, while 25 (20.8%) of female patients with GC tested positive (p=0.91).

For CMV DNA, the highest positive rate was 37 (30.8%) and the lowest positive rate was 12 (10%) for the high and low levels of serum midkine, respectively. High association was found between S-MK levels and CMV infection among study groups (p= 0.001) (Table 3).

Table 3. Association between human cytomegalovirus (CMV) infection and demographic features.

Study variables		Total	CMV-DNA (PCR) Positive *n* = 49 (40.8%)	CMV-DNA (PCR) Negative *n* = 71 (59.2%)
Study groups	Control	30 (25%)	5 (4.2%)	25 (20.8%)
	Gastric precancerous lesions	45 (37.5%)	9 (7.5%)	36 (30%)
	Gastric adenocarcinomas	45 (37.5%)	35 (29.2%)	10 (8.3%)
	p-value	*< 0.001*		
Sex	Male	61 (49.2%)	24 (20%)	37 (30.8%)
	Female	59 (50.8%)	25 (20.8%)	34 (28.3%)
	p-value	*0.91>0.05*		
Age categories	< 50 years	63 (52.5%)	26 (21.7%)	37 (30.8%)
	≥ 50 years	57 (47.5%)	23 (19.2%)	34 (28.3%)
	p-value	*0.73>0.05*		
*S-MK levels	High	52 (43.3%)	37 (30.8%)	15 (12.5%)
	Low	68 (56.7%)	12 (10%)	56 (46.7%)
	p-value	*< 0.001*		

*S-MK= Serum midkine, *p-value* > 0.05 significant, <0.05 non-significant, <0.001 highly-significant.

Relationship between HCMV infection and Clinicopathological features of GC

Table 4 showed that the positive rate of CMV DNA was significantly higher in the GC group than in the other study group, as shown previously. The highly significant distribution of positive CMV DNA in pathological stages was detected in stage III 21 (46.7%) and stage IV 10 (22.2%) (p= 0.001). The presence of metastases was directly associated with positive CMV infection in 23 (51.1%). It was also observed that the number of positive PCR cases was higher among patients with diffuse tumor type, as 29 (64.4%) (p= 0.001). The positivity for CMV in these more severe cancer cases was 26 (57.8%) in advanced stages of T3 and T4 with lymph node metastasis in 22 (48.9%) of cases, 20 (44.4%) for CMV DNA positive GC compared with 2 (4.4%) for negative results, the difference was statistically significant (p=0.03).

Table 4. Relationship between cytomegalovirus (CMV) and clinicopathological features of GC.

Study variables		Total	CMV-DNA (PCR) Positive *n* = 35 (77.8%)	CMV-DNA (PCR) Negative *n* = 10 (22.2%)
Tumor depth	Early (*T1, T2)	15 (33.3%)	9 (20%)	6 (13.3%)
	Advanced (T3, T4)	30 (66.7%)	26 (57.8%)	4 (8.9%)
	p-value		*0.04*	
**N factor	N0	23 (51.1%)	15 (33.3%)	8 (17.8%)
	N1	22 (48.9%)	20 (44.4%)	2 (4.4%)
	p-value		*0.03*	
***M factor	M0	19 (42.2%)	12 (26.7%)	7 (15.6%)
	M1	26 (57.8%)	23 (51.1%)	3 (6.7%)
	p-value		*0.04*	
Tumor stages	I	3 (6.7%)	0 (0%)	3 (6.7%)
	II	9 (20%)	4 (8.9%)	5 (11.1%)
	III	23 (51.1%)	21 (46.7%)	2 (4.4%)
	IV	10 (22.2%)	10 (22.2%)	0 (0%)
	p-value		*<0.001*	
Tumor grades	Differentiated	29 (64.4%)	20 (44.4%)	9 (20%)
	Undifferentiated	16 (35.6%)	15 (33.3%)	1 (2.2%)
	p-value		*0.05*	
Lauren types	Diffuse	32 (71.1%)	29 (64.4%)	3 (6.7%)
	Intestinal	13 (28.9%)	6 (13.3%)	7 (15.6%)
	p-value		*0.001*	
Tumor sites	Antrum	12 (26.7%)	9 (20%)	3 (6.7%)
	Body	16 (35.6%)	13 (28.9%)	3 (6.7%)
	Cardia	17 (37.8%)	13 (28.9%)	4 (8.9%)
	p-value		*0.09*	

*T= tumor, ** N= Nodes, ***M= Metastasis, *p-value* > 0.05 significant, significant, <0.001 highly-significant

Highly significant differences (p=0.001) of S-MK levels in T3/T4 phases, which were higher (519.47±171.28 pg/mL) than those in T1/T2 phases (511.43±118.31 pg/mL), with a marked correlation between high S-MK level and nodal involvement (525.39±158.32 pg/mL), or distant metastasis (519.42±170.25 pg/mL). Furthermore, levels of S-MK gradually increased along with the stage, which was higher in stage III/IV, as (531.70±138.99 pg/mL) and (575.63±122.65 pg/mL) compared to stages I/II. According to Lauren's criteria, 32 (71.1%) of the diffuse tumors had high S-MK levels(537.41±169.79 pg/mL), compared with the intestinal type (p=0.08). Finally, the distribution of S-MK levels across stomach tumors (antrum, body, and cardia) showed no significant differences (p=0.7), as shown in Table 5.

Table 5. Relationship between S-MK levels and clinicopathological features of gastric cancer (GC)

Gastric adenocarcinoma variables		N (%)	S-MK (pg/mL)	*p-value*
Tumor depth	Early (*T1,T2)	15 (33.3%)	511.43±118.31	
	Advanced (T3,T4)	30 (66.7%)	519.47±171.28	
**N factor	N0	23 (51.1%)	503.75±143.30	
	N1	22 (48.9%)	525.39±158.32	
***M factor	M0	19 (42.2%)	509.93±106.88	
	M1	26 (57.8%)	519.42±170.25	*< 0.001*
Tumor stages	I	3 (6.7%)	465.86±93.33	
	II	9 (20%)	501.61±181.18	
	III	23 (51.1%)	531.70±138.99	
	IV	10 (22.2%)	575.63±122.65	
Tumor grades	Differentiated	29 (64.4%)	402.55±64.39	
	Undifferentiated	16 (35.6%)	540.84±153.35	
Lauren types	Diffuse	32 (71.1%)	537.41±169.79	*0.08*
	Intestinal	13 (28.9%)	504.58±141.15	
Tumor sites	Antrum	12 (26.7%)	510.40±172.93	
	Body	16 (35.6%)	498.63±126.138	*0.7*
	Cardia	17 (37.8%)	536.97±162.23	

*T= tumor, ** N= Nodes, ***M= Metastasis, *p-value* > 0.05 significant, significant, <0.001 highly-significant

DISCUSSION

Gastric cancer is one of the most common types of cancer worldwide, ranking as the fifth most common cancer and the third leading cause of cancer-related deaths. Its prevalence varies significantly by geographic region, with the highest rates observed in Eastern Asia, including countries like Japan, Korea, and China. In Iraq (2020), gastric cancer accounted for approximately 3.7% of all cancer cases, with an incidence rate of about 2.8 per 100,000 population. This variation in incidence is partly attributed to differences in dietary habits, particularly the consumption of certain preserved and salted foods (Ilic & Ilic, 2022). In addition, gastric cancer cells have been found to express higher levels of midkine compared to normal gastric tissues. This overexpression is believed to be associated with increased tumor growth, angiogenesis, and metastasis. Midkine has shown promise as a potential biomarker for gastric cancer detection. Studies have indicated that elevated midkine levels in the

blood may be associated with the presence of gastric cancer (Shin et al., 2020). Therefore, measuring midkine serum levels could aid in the early detection and diagnosis of gastric cancer, enabling timely intervention and treatment, as the current study demonstrates a significant association of midkine levels with gastric cancer parameters (Table 5).

On the other hand, midkine serum levels have been studied as a prognostic indicator for gastric cancer patients. Higher midkine levels have been associated with more aggressive tumor behavior, advanced disease stages, and a poorer prognosis, as shown in Table 2, which showed different and gradual S-MK serum levels among the studied groups (Siregar et al., 2022). Gastric cancer patients with elevated midkine levels in their serum may have a higher risk of disease progression and reduced overall survival. Monitoring midkine levels during and after treatment could also be valuable in assessing treatment response and disease progression. Changes in midkine serum levels over time may provide insights into the effectiveness of therapeutic interventions and help clinicians make informed decisions about treatment adjustments (Ito et al., 2019).

It is important to note that while midkine serum levels show promise as a potential biomarker for gastric cancer, further research is needed to fully establish its clinical utility. As of the last update in September 2021, midkine was not yet a routine diagnostic or prognostic tool in clinical practice (Siregar et al., 2022).

Furthermore, CMV is a common virus belonging to the herpesvirus family. It can infect people of all ages and is usually asymptomatic or causes mild flu-like symptoms in healthy individuals. However, CMV can become a significant concern for people with weakened immune systems, such as those with HIV/AIDS, organ transplant recipients, or individuals undergoing immunosuppressive therapy (Griffiths & Reeves, 2021).

As of the last update in September 2021, there was limited research available on the direct relationship between CMV infection and midkine levels in gastric cancer (Liu et al., 2020). Until now, there has been no direct and dependable evidence of the relationship between human CMV and GC in spite of our data finding a significant association between CMV DNA detection and gastric cancer clinicopathological characteristics (Table 4).

However, there is some research on the association between CMV infection and specific cancer-related proteins or cytokines in other types of cancers (Muhsin & Abbas, 2016; Muhsin et al., 2019). Some studies have suggested that CMV infection might influence the expression of certain proteins involved in cancer development and progression.

The connection between CMV infection and stomach cancer is still poorly understood. CMV nucleic acids and proteins have been detected in human colorectal cancer, although they are not associated with cell immortalization or increased proliferation *in vitro.* Additionally, Zhang et

al. (2017) demonstrated that gastric cancer tissue had a higher prevalence of CMV infection than in healthy individuals' normal gastric tissue. However, it remains unclear whether CMV infection was the cause or a byproduct of carcinogenesis (Herbein, 2018). Finally, it's worth noting that the field of cancer research is continually evolving, and studies with new findings may be needed. Therefore, it is crucial to conduct medical studies concerning these issues. Finally, the findings are based on an Iraqi cohort; therefore, generalizability to other regions with different gastric cancer epidemiology, CMV prevalence, or dietary/lifestyle factors may be limited. Validation in larger, multi-center cohorts is recommended.

This study has several limitations. The sample size, although adequate for exploratory analysis, was relatively small and drawn from two centers, which may limit external validity. Potential confounders, including *Helicobacter pylori* infection status and lifestyle factors, were not evaluated. Additionally, the cross-sectional design prevents establishing causality. These sources of bias and imprecision should be addressed in future longitudinal investigations.

Our data clearly show the prevalence of human CMV in GC patients and conclude that midkine serum levels may serve as a prognostic and monitoring signal for gastric cancer patients. Human CMV infection in malignant tissues may be connected with carcinogenesis or the development of GC as a cofactor.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to disclose.

REFERENCES

1. Ajani JA, D'Amico TA, Almhanna K, Bentrem DJ, Chao J, Cooke D, Corvera C, Prajnan D, Enzinger PC, Enzler T, Fanta P, Farjah F, Gerdes H, Gibson MK, Hochwald S, Hofstetter WL, Ilson DH, Keswani RN, Kim S, Kleinberg LR, Klempner SJ, Lacy J, Ly QP, Matkowskyj KA, McNamara M, Mulcahy MF, Outlaw D, Park H, Perry KA, Pimiento J, Poultsides GA, Reznik S, Roses RE, Strong VE, Su S, Wang HL, Wiesner G, Willett CG, Yakoub D, Yoon H, McMillian N, Pluchino LA. Gastric cancer, version 2.2022, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw 20:* 167-192, 2022.
2. Griffiths P, Reeves M. Pathogenesis of human cytomegalovirus in the immunocompromised host. *Nat Rev Microbiol 19:* 759-773, 2021.
3. Hajian-Tilaki K. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. *Caspian J Int Med 4:* 627, 2013.
4. Herbein G. The human cytomegalovirus, from oncomodulation to oncogenesis. *Viruses 10:* 408, 2018.
5. Ilic M, Ilic I. Epidemiology of stomach cancer. *World J Gastroenterol 28:* 1187, 2022.

6. Ito M, Oshima O, Yajima S, Suzuki T, Nanami T, Shiratori F, Funahashi K, Shimada H. Diagnostic impact of high serum midkine level in patients with gastric cancer. *Ann Gastroenterol Surg 3:* 195-201, 2019.

7. Ji L, Gu L, Zhang X, Wang J. Evaluation of serum midkine and carcinoembryonic antigen as diagnostic biomarkers for rectal cancer and synchronous metastasis. *Transl Cancer Res 9:* 2814, 2020.

8. Liu X, Lin K, Huang X, Xie W, Xiang D, Ding N, Hu C, Shen X, Xue X, Huang Y. Overexpression of the human cytomegalovirus UL111A is correlated with favorable survival of patients with gastric cancer and changes T-cell infiltration and suppresses carcinogenesis. *J Cancer Res Clin Oncol 146:* 555-568, 2020.

9. Lv YL, Han FF, An ZL, Jia Y, Xuan LL, Gong LL, Zhang W, Ren LL, Yang S, Liu H, Liu LH. Cytomegalovirus infection is a risk factor in gastrointestinal cancer: A cross-sectional and meta-analysis study. *Intervirol 63:* 10-16, 2020.

10. Muhsin JM, Abbas SH. Evaluation of the possible role of HPV16, CMV and EBV in cervical carcinoma progression using in situ hybridization technique. *Diyala J Med 11:* 37-43, 2016.

11. Muhsin JM, Yousif SO, Hadi AM, Rawdhan AR Determination of the cytomegalovirus (CMV) infection role with the disturbances of immunoglobulin E (IgE) and interleukin-33 (IL-33) concentrations in the pathogenesis of asthma and atherosclerosis in a sample of Iraqi patients. *J Pure Appl Microbiol 13:* 1003-1010, 2019.

12. Parija SC. Herpesviruses. In: *Textbook of Microbiology and Immunology.* Springer: Berlim, 2023. p.751-773.

13. Schober P, Vetter TR. Chi-square tests in medical research. *Anesth Analg 129:* 1193, 2019.

14. Shin DH, Jo JY, Kim SH, Choi M, Han C, Choi BK, Kim SS. Midkine is a potential therapeutic target of tumorigenesis, angiogenesis, and metastasis in non-small cell lung cancer. *Cancers 12:* 2402, 2020.

15. Siregar J, Darmadi D, Ganie RA. Association between serum midkine level and gastric precancerous lesion in patients with gastritis. *Med Arch 76:* 368, 2022.

16. Varani S, Landini MP. Cytomegalovirus-induced immunopathology and its clinical consequences. *Herpesviridae 2:* 1-14, 2011.

17. Zhang L, Guo G, Xu J, Sun X, Chen W, Jin J, Hu C, Zhang P, Shen X, Xue X. Human cytomegalovirus detection in gastric cancer and its possible association with lymphatic metastasis. *Diagn Microbiol Infect Dis 88:* 62–68, 2017.