Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5127 - 5137

Received 15 May 2021; Accepted 20 May 2021.

The Gene Expression of *ADAM9* in patients with gastric cancer in Iraq

Raghad Khalid Mwafaq [1*], Amal Khudair Abbas[2], Leith Abdul Hussein Abdullah[3]

[1]Department of biology, College of Science, University of Baghdad. Iraq

[2] Department of biology, College of Science, University of Baghdad. Iraq

[3]Consultant gastroenterologist and heptologist, Baghdad Hospital, Medical City, Baghdad, Iraq

raghad.khaled1102a@Sc.uobaghdad.edu.iq[1*]/ORCID ID: https://orcid.org/0000-0002-8537-986X

amal.khudair@Sc.uobaghdad.edu.iq[2]/ ORCID ID: https://orcid.org/0000-0002-5712-7868

leith75@ymail.com[3] / ORCID ID: https://orcid.org/0000-0002-7566-1896

Abstract

ADAM9 disintegrin and metalloproteinase domain 9 gene, has been institute, and characterize overexpression, and dysregulation in a different of solid tumors. Objective: Evaluation the Gene Expression of *ADAM9* in gastric cancer patients in Iraq. Furthermore, found the correlation between *ADAM9*expression, and clinic-pathological parameters.50 randomly patients FFPE blocks of stomach tissue (10 normal & 40 patients GC) were collected from the Gastroenterology and Hepatology Teaching Hospital. *ADAM9* Gene Expressionhas been achieved by using qPCR. Statistical analysis accomplished by SPSS system at ($P<0.05$). This study indicated that there were a significant differences between control and patients, also there was a significant correlation in invasion depth, lymph node involvement, and stages in patients, whereas there was non- significant association between the age, grade, gender, and histopathological type of patients in the expression of *ADAM9*. These results indicated that *ADAM9* could be a functional target for advanced gastric cancer therapeutic.

Keywords: *ADAM9*, Clinicopathological parameters, FFPE, Gastric cancer, Gene Expression.

Introduction

Gastric carcinoma (GC) comprises a universal health issue. It is a disease with high aggressive and heterogeneous nature. It is one of the most prevalent reasons of cancer related death and takes advantage of an important encumbrance on international health sponsor [1]. In Iraq malignant neoplasm's represented the second leading cause of death. Gastric cancer trend demonstrated an instant rise after 2007; it is a fifth of eighteenth cancers in Iraq [2]. GC is the third main reason of cancer-related mortality in the world, leading round about 783,000 deaths in 2018, and over 1,000,000 new cases of gastric carcinoma per year [3]. The Lauren classification is the most communal classification of GC. It includes three main subtypes: intestinal, diffuse and mix [4], which they differ in many properties, such as: clinical characterize, genetics, morphology, epidemiology and development features [5].*ADAM9* disintegrin and metalloproteinase domain 9, or meltrin-γ, also known as metalloprotease/disintegrin/cysteine-rich protein 9 (MDC9), was first recognize in breast cancer in 1996 [6], has been institute, and characterize overexpression, and dysregulation in a different of solid tumors. It proposes that a main molecule includes in tumorigenesis in glioma, prostate, colon and breast cancer, which suggest *ADAM9* as an important molecule involved in tumorigenesis [7], [8]. Nevertheless, the role of gene in gastric cancer is remaining elusive, and merit to be explained [9]. It is greatly expressed in human tissues, and exhibits a plentiful rise in pathological cases [10]. Gene location in human: 8p 11.23, exon count: 22 and 4447BP full length [11].It has several domains structures such as: The long ADAM9-L (110 and 84) KDa. The short ADAM9-S (55) KDa. ADAM9-S lacks exon 12 from the ADAM9-L genetic sequence [12]. Moreover, *ADAM9*, its N-terminus includes a signal sequence that controller the secretory pathway of it to passage to the cell surface, and the

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5127 - 5137

Received 15 May 2021; Accepted 20 May 2021.

transmembrane domain that confirm it on the cell membrane [13].ADAM proteins have been reported in many biological pathways such as: development, fertility, vascular endothelial cell function, inflammation, immunity, signaling transduction, neurodegenerative disease [14], [15], [16], [17]. Objective: To estimate *ADAM9* expression in patients with gastric cancer, and control. Furthermore, found the correlation between *ADAM9*expression, and clinicopathological parameters as: gender, age, invasion depth, histopathological type, involvement of lymph nodes, grade and stages of GC.

Materials and methods

Samples collection

The current research is a retrospective study from January 2018 to December 2020. The total numbers of samples were 50 cases. 40 samples of gastric cancer patients formalin-fixed paraffin embedded blocks (FFPE) have been obtained randomly from surgically resected specimens in Gastroenterology and hepatology Teaching Hospital, Medical City/ Bagdad, with its reports (no chemotherapy received by these patients) after getting the official agreement from Iraq Ministry of Health, and Department of Medical City/ Baghdad. Each report contains clinic-pathological parameters (age, gender, histopathological type of tumor, grade of tumor, depth invasion of tumor, lymph nodes involvement, tumor stage/ TNM), which were diagnosis by pathological doctors of hospital. 10 samples of normal stomach tissue have been selected randomly from patients undergo (Sleeve gastrectomy) by private hospitals. These patients cases were classified depend on Lauren classification [4].

Gene Expression study

Primers

Primers for Gene Expression have been listed depended on [18],[19] in (Table 1) as follows:

Table (1): primers for Gene Expression

Name of gene	Primer sequence	Reference
ADAM9	F- 5'-AGTGGCGGGAAAAGTTTCTT-3' R-5'-CCAGCGTCCACC-3'	[18]
Beta-actin (housekeeping gene)	F-5'-ATGATATCGCCGCGCTCGTC-3' R-5- CGCTCGGTGAGGATCTTCA-3,	[19]

The study of Gene Expression of ADAM9 accomplished as follows:

RNA extraction

By using Qiagen RNeasy extraction kit (RNeasy Mini Kit (217504), Germany), total RNA was extracted from cancerous and non-cancer gastric tissues depended on manufacture protocol as follows: A scalpel used to trim excess paraffin off the sample block, which cut about (5–20) µm thick. It placed in a 1.5 ml microcentrifuge tube, 320 µl of xylene was added, and vortex vigorously for (10 s). Tube incubated at 56°C for 3 min, and then allowed to cool at room temperature (15–25°C). 240 µl of Buffer PKDwas added and mixed by vortexing then it was centrifuged for 1 min at 11,000 x g.10 µl proteinase K was added to the lower, clear phase and mixed gently, incubated at 56°C for 15 min, and then at 80°C for 15 min.The lower, uncolored phase transfered into a new 2 ml microcentrifuge tube, which incubated on ice for 3 min. and

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5127 - 5137

Received 15 May 2021; Accepted 20 May 2021.

then centrifuged for 15 min at 20,000 x g.The supernatant transferred to a new microcentrifuge tube. DNase Booster Buffer added approximately 25 µl, and 10 µl DNase I stock solution mixed by inverting the tube. Microcentrifuge tube incubated at room temperature for 15 min.500 µl Buffer RBC added to adjust binding conditions, and mixed the lysate thoroughly. 1200 µl ethanol (100%) added to the sample, and mixed well. 700 µl of the sample Transferred, including any precipitate that may have formed, to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). The lid closed gently, and microcentrifuge tube centrifuged for 15 s at ≥8000 x g. 500 µl Buffer RPE added to the RNeasy MinElute spin column, the lid closed gently, and centrifuged for 15 s at ≥8000 x g. The flow-through discarded. 500 µl Buffer RPE added to the RNeasy MinElute spin column, the lid closed gently, and centrifuged for 2 min at ≥8000 x g. The collection tube discarded with the flow-through. The RNeasy MinElute spin column placed in a new 2 ml collection tube, the lid of the spin column opened, and centrifuged at full speed for 5 min, the collection discarded tube with the flow-through. The RNeasy MinElute spin column placed in a new 1.5 ml collection tube. 14–30 µl RNase-free water added directly to the spin column membrane. The lid closed gently, and centrifuged for 1 min at full speed to elute the RNA.

RNA quantitation by Qubit 4.0

The Qubit® working solution was prepared by diluting the Qubit® RNA HS [Qubit™ RNA HS Assay Kit(Q32852), ThermoFisher(USA)] Reagent 1:200 in Qubit® RNA HS buffer. The volume 190 µL from Qubit® working solution has been added to each tube designed to be as a standard, then 10 µl from each provided standard solution has been added into same tubes, then vortexed.The Qubit® working solution as 197 µL has been added to each tube prepared for sample and then 3 µL of sample has been added individually.All composition has been vortexed and incubated at room temperature for 3 mins.Standards tubes have been inserted in Qubit instrument for creating concentration curve.Tubes for samples have been added one by one to read the concentration for miRNA in each sample.

RT-qPCR protocol

This process divided into two phases, the first is done through synthesis of cDNA from RNA through specific primer for fas, fasl and beta actin transcripts, and protoscript cDNA synthesis kit [ProtoScript® First Strand cDNA Synthesis Kit(E6300S), NEB (UK)]. This procedure has performed through steps: 5 µl from each extracted total RNA sample added into new PCR tube. Protoscript reaction mix that contains dNTPs, buffer and other essential components added as 10ul for each sample.MuLV Enzyme then added into reaction as 2ul per sample.2 µl of oligoT, and the volume completed up to 20ul by adding 1ul, and then this mixture was incubated for 60 min at 42 Cº by using thermocycler as in (Table 2), and this followed by 80 Cº.

Table (2): Thermocycling protocol for cDNA synthesis

Step	Temperature	Time
Primer annealing	30 Cº	10 min
cDNA synthesis	42 Cº	60 min
Heat inactivation	80 Cº	5 min

The cDNA product quantification also done through Qubit 4.0. The second section of this protocol it's done by choosing the cDNA sample from patient, and control at the same run, for each sample there are three PCR tubes, one tube for each gene, fas, fasl and beta- actine which is consider as a house keeping

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5127 - 5137

Received 15 May 2021; Accepted 20 May 2021.

gene in this study. The detection of quantity based on fluorescent power of SyberGreen. The reaction mix composed from component with their quantity as mentioned in (Table 3) below:

Table (3): The reaction mix composed from component with their quantity

Component	20ul Reaction
Luna Universal qPCR Master Mix [(M3003S), NEB (UK)]	10ul
Forward primer (10 µM)	1ul
Reverse primer (10 µM)	1ul
Template DNA	5ul
Nuclease-free Water	3ul

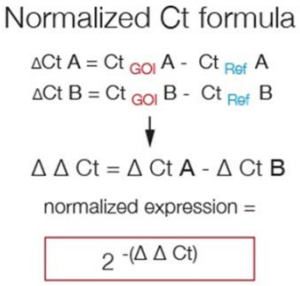
Quickly spin for PCR tubes to remove the bubbles, and collect the liquid (1 minute at 2000g), and then the program for Real-Time PCR was setup with indicated thermocycling protocol as shown in (Table 4).

Table (4): Thermocycling protocol for Real-Time PCR

Cycle Step	Temperature	Time	Cycles
Initial Denaturation	95 Cº	60 seconds	1
Denaturation	95 Cº	15 seconds	
Extension	60 Cº	30 seconds (+plate read)	40-45
Melt Curve	60-95 Cº	40 minutes	1

Results calculation

The results were calculated, and analyzed by Livak formula:



Ct refer to stands for the cycle threshold of sample, it is the cycle number where the fluorescence generated.

ΔCt = Ct (gene of interest) – Ct (housekeeping gene)

ΔΔCt = ΔCt (sample) – ΔCt (control) [20]

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5127 - 5137

Received 15 May 2021; Accepted 20 May 2021.

Statistical analysis

Collected data analysis was accomplished by (statistical package for social science/ SPSS version- 24 software/IBM), Chi square (x2) used to estimate the correlation between clinic-pathological correlations, and (t-test, and ANOVA test) used for the data of Gene Expression, (P- values) valuable at the accepted level of significance in 0.05 ($p<0.05$ was considered significant) [21].

Results

The results of 50 cases for this study referred that males total numbers in control group were 2 (20%) statuses, while females were 8 (80%) statuses, with male: female (M: F) ratio 1:4 (the majority of statuses from females). In patients with gastric cancer group, the number of males were 24 (60%) cases, while females number were 16 (40%) cases, and 3:2 in (M: F) ratio (the majority cases from males). Age divided into two age group equal or less than 50 years, and more than 50 years. Control group age ranged between (19-50) years for 10 (100%) statuses, which were within the age group equal or less than 50 years with mean age (33.9 ± 10.027), and the age of patients with gastric cancer which were ranged from (19-83) years with mean (55.325 ± 15.423). Patients number of equal or less than 50 years were 16 (40%) cases with mean (39.875 ± 9.493), and the number of patients in more than 50 years were 24 (60%) cases with mean age (65.625 ± 8.234). Histopathological subtype distributed in: intestinal type: patients numbers were recorded 23 (57.5%) cases. Diffuse type: 15 (37.5%) cases total number of patients, whereas mixed type: patients were registered 2 (5%) cases. The most cases for grade were moderately differentiated which were registered 25 (62.5%) cases, while poorly differentiated were 15 (37.5%) cases in total number of patients. Also, the current study was showed that gastric cancer invasion (subserosa) in about 15 (37.5%) cases. In invasion (serosa) total patients were 18 (45%) cases, while (muscularis propria) invasion were 7 (17.5%) cases in total patients. The total number of patients that diagnosed with lymph node involvement was about 32 (80%) cases, whereas patients without lymph node involvement total number were 8 (20%) cases. Invasive depth and lymph node metastasis (TNM) were used to distribute gastric cancer patients into advanced stages (III & IV), and early stage (II). Advanced stages (III & IV) total patient numbers were 27 (67.5%) cases. In (III) stage the total number of patients were about 12 (30%) cases, whereas IV stage total patient numbers were 15 (37.5%) cases. 13 (32.5%) cases were the total number of patients in early stage (II) as fellow in (Table 5):

Table (5): Study groups distribution depends on clinicopathological parameters

Clinicopathological Parameters	Findings		Frequency (%)
Gender	Control	Male	2 (20%)
		Female	8 (80%)
	Patients	Male	24 (60%)
		Female	16 (40%)
Age	≤ 50	Control	10 (100%) with mean (33.9±10.027)
		Patients	16 (40%) with mean (39.875±9.493)
	> 50	Control	–
		Patients	24 (60%) with mean (65.625±8.234)
Histopathological types	Patients	Intestinal	23 (57.5%)
		Diffuse	15 (37.5%)
		Mix	2 (5%)
Grade of tumor	Patients	Moderately	25 (62.5%)
		Poorly	15 (37.5%)
Invasion depth of tumor	Patients	pT2	7 (17.5%)
		pT3& pT4	33 (82.5%)
Involvement of lymph node	Patients	N0	8 (20%)
		N1,2&3	32 (80%)
Stage of GC (TNM)	Patients	II	13 (32.5%)
		III&IV	27 (67.5%)

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5127 - 5137

Received 15 May 2021; Accepted 20 May 2021.

The Gene Expression of *ADAM9*

The amplification of *ADAM9* by qPCR of some study group (control and patients) illustrated in fig. (1, A), which show Ct values of these groups. Furthermore, the fig. (1, B) show the different fold gene expression of *ADAM9* in control and patients.

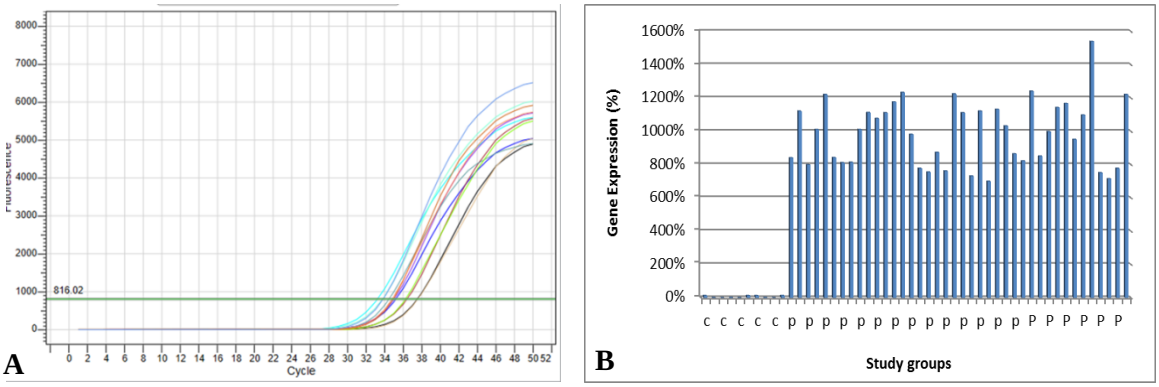


Figure (1): (A) *ADAM9* amplification by qPCR samples included some study groups. Ct values ranged from (32-36). The photograph was taken directly from mic- qPCR machine. (B)Illustrates the Gene Expression of *ADAM9* in control (c) and patients (p) with gastric cancer.

The Gene Expression of a disintegrin and metalloproteinases (*ADAM9*) in control and patients with gastric cancer.

This study investigated the Gene Expression of *ADAM9* in study groups which contain (control and patients), the result indicated that this gene was not expressed in control group (n=10) with mean of fold Gene Expression (0.061 ± 0.019), and 6.0003 in variance, whereas it expressed in patients group (n=40) with mean (9.879 ± 0.310), and 188.028 in variance. Statistically, these differences made a significant association at $P < 0.05$, $P= 0.0001$ as showed in (Table 6), and fig. (2).

Table (6): The Gene Expression of ADAM9 in control and patients with gastric cancer.

Group of study	Fold of Gene Expression		P-value
	Mean ±SE	Variance	
Control (n=10)	0.061 ± 0.019	6.0003	P=0.0001*
Patients (n=40)	9.879 ± 0.310	188.028	

***t-test P-value is significant (P<0.05)**

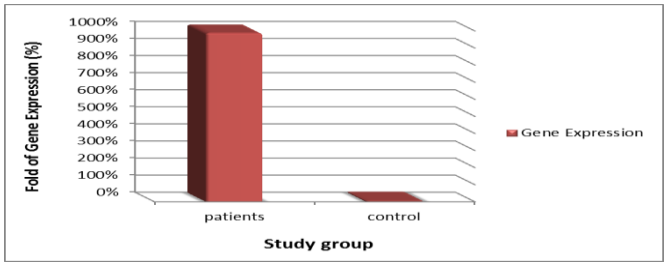


Figure (2): Graph illustrates the Gene Expression of *ADAM9* in control and patients with gastric cancer.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5127 - 5137

Received 15 May 2021; Accepted 20 May 2021.

Patients samples distribution of fold Gene Expression of a disintegrin and metalloproteinases (*ADAM9*) depend on clinicopathological parameters

(Table 7) Show the fold of Gene Expression of *ADAM9* correlated with clinic-pathological parameters. This study donate that (60%) with mean (9.746 ±0.337) of total numbers in gastric cancer patients were in males, which recorded in 24 cases, and (40%) 16 cases, with mean (9.211±0.70) for female, statistically, no significant association between gender and the fold of Gene Expression, P- value were larger than 0.05 at ($P < 0.05$). Patients age ranged from (19-83) years, patients from less or equal 50 years were recorded (40%), with mean (8.996±0.670), and (60%), with mean (9.901±0.350) in patients more than 50 years. P-value = 0.184larger than 0.05 ($P > 0.05$) so there was not a significant association between age and the expression of this marker. The current study showed that the total number of patients with intestinal type positive expressed were (57.5%), diffuse were (37.5%), and (5%) in mixed type, with mean (10.04±0.0092, 9.72±0.059, 8.69±0.012) respectively. No significant association at ($P < 0.05$) P=0.151. There was about (62.5%) cases with gastric cancer at moderately differentiated grade with mean (9.975±0.387), which were more than poorly differentiated grade, which recorded (37.5%), (8.794±0.622) in mean, but there was not a significant correlation p=0.779. The fold of Gene Expression of *ADAM9*in invasion depth were recorded (20%), and (7.556±0.061) mean in pT2, and (80%), and (20.671±0.060) mean in p T (3 &4), a significant association statistically at $P < 0.05$, p=0.004. Also, this study indicated that there were (80%), (7.843±0.209) of *ADAM9* fold of Gene Expression in patients involve lymph nodes (N1, N2&N3), whereas in patients without involvement of lymph nodes (N0) patients were (20%), (9.954±0.393) mean. Statistically, these differences made a significant association between involvement of lymph nodes and the expression of this gene, p=0.012. The highest records of GeneExpression were in advanced stage (III&IV) of gastric cancer in (67.5%)with mean (20.432±0.738), while it was (32.5%) with mean(7.800±0.129) in early stage. Statistically, these differences made a significant association between stage of gastric cancer, and the expression of this gene at $p < 0.05$, p= 0.0003.

Table (7): The fold of Gene Expression of *ADAM9* correlated with clinicopathological parameters

Clinicopathological parameters		Fold of Gene Expression of *ADAM9*			P-value
		Number (ratio)	Mean%	Mean ±SE	
Gender	Male	24 (60%)	975	9.746 ±0.337	P=0.480*
	Female	16 (40%)	921	9.211±0.70	
Age	≤ 50	16 (40%)	899.6	8.996±0.670	P= 0.184*
	> 50	24 (60%)	990.1	9.901±0.350	
Histopathological subtype	Intestinal	23 (57.5%)	1004	10.04±0.0092	P= 0.151**
	Diffuse	15 (37.5%)	972	9.72±0.059	
	Mix	2 (5%)	869	8.69±0.012	
Grade of tumor	Moderately	25 (62.5%)	997.5	9.975±0.387	P= 0.779*
	Poorly	15 (37.5%)	879.4	8.794±0.622	
Invasion depth of GC	pT 2	8 (20%)	755.6	7.556±0.061	P= 0.004*
	pT3 & pT4	32 (80%)	206.7	20.671±0.060	
Involvement of lymph node	N0	8 (20%)	784.3	7.843±0.209	P= 0.012*
	N1,2,&3	32 (80%)	995.4	9.954±0.393	
Stage of GC (TNM)	II	13 (32.5%)	780.0	7.800±0.129	P= 0.0003*
	III&IV	27 (67.5%)	2043.2	20.432±0.738	

***t-test, and **ANOVA test, P-value is significant at (P<0.05)**

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5127 - 5137

Received 15 May 2021; Accepted 20 May 2021.

Discussion

In Iraq malignant neoplasm's represented the second leading cause of death. Gastric cancer trend demonstrated an instant rise after 2007; it is a fifth of eighteenth cancers in Iraq [2]. GC comprises a universal health issue. It is a malignancy disease with high aggressive nature. It is one of the most prevalent reasons of cancer related death and takes advantage of an important encumbrance on international health sponsor [1]. Gastric cancer treatment is restricted because of its heterogeneity and genetic complicated [22] so find of special biomarkers is important for management the development of gastric cancer, and identified the effective treatments for patients [23]. The current study indicated that the total number of male more than female, and the number of older patients (more than 50 years) more than younger (equal or less than 50 years), as in the study of Lou *et al*. [24] which is show that men is higher incidence than women. Furthermore, there is a maximal sex differences in older age. Moreover, as in the Globocan 2012 report, standardized rates of age of gastric cancer was twice more in men than in women [25]. The study of Radkiewicz *et al*. [26] is demonstrated that rise of risk for gastric cancer is associated with male sex, the portion of cancer explicated by factors correlated to male sex is huge, and males suffer poorer survival in most cancer sites. A probable exposition is either the preventative impact of estrogen in women, or other effects as diversity in diet and occupational exposure may participate to rise gastric cancer incidence in males [27],[28]. Many studies mention that the intestinal type is the general type with rising the risk of adenocarcinoma, in age range (55-80) years, commonly appear in male more than female in ratio (2:1) [29],[30]. Also, in [31] author reminded that (54%) of cases were intestinal subtype, which located in distal stomach (non- cardia). All these results are corresponded with this study, which intestinal type was the highest ratio compare with diffuse and mix type. The study recorded that moderately differentiated was the high ration than poorly, which is similarity in [32], in contrast the study of Zhang *et al*. [33] which found that poorly differentiated was the domain differentiated. Advanced stage (III&IV) depend on TNM staging was the highest ratio than early stage (II) this result agree with Chen *et al*. and Lee *et al*.[34],[35].*ADAM9* is a one of ADAM family anchored to the membrane member's.Many reports have mentionedthat there are high expressions of *ADAM9* in GC. It associated with cancer proliferation and invasion [36], [37].It was a significant upregulated in patients with gastric cancer compared with normal tissue on mRNA and protein level as show in (Table 6). As in (Table 7) this study declared that there was not a significant association between age and the expression of the gene which is match with Grutzmann*et al.*[38]. In the study of Wang *et al*. [39] demonstrated that there were a significant correlated in invasion depth of tumor, involvement of lymph nodes and stages of GC, and no significant association in age, gender, and grade of GC and the fold of Gene Expression of this gene, these results matched with current study as in (Table 7).*ADAM9*havean essential in somesignaling pathwayroles, in whichtransportation of acquaintancecould promptseveralexacerbations of inconvertible in disease [40].There aremanyconstructions about *ADAM9* role in cancer advancement. It intermediates the secretion of growth factor ligands, which regulate the signaling of EGFR. In the gastric, the EGFR trans-activation accomplishby peptides as:(gastrin, angiotensin II, bradykinin, bombesin, or substance P)that intermediated by EGFR-ligandsshedding by ADAMs [41].Furthermore, ittrans-membrane protein-derived ligands releasesas:(heparin-binding-EGF, amphiregulin, TGFα, and TNFα) [42]. In the study of Kim *et al*. [18] found that knock-down of *ADAM9* blocks EGFR phosphorylation, and down-stream ERK activation that RAV-18 inhibited tumor growth, in line with EGFR, and ERK phosphorylation in vivo. So, suppression of EGFR ligand shedding by ADAM9 with followingreduced the activity of EGFR, and down-stream signaling might be a techniqueagainst the anti-tumor activity of RAV-18.

Conclusion:*ADAM9*could be speculating as a prospect target for GC prohibition, therapeutic treatment, anddiagnosis.

Received 15 May 2021; Accepted 20 May 2021.

Acknowledgements

All thanks and appreciation to all members in Gastroenterology and Hepatology Teaching Hospital, and some private hospitals in Baghdad for all help to accomplish this study.

References

[1] Gao, J. P., Xu, W., Liu, W. T., Yan, M. & Zhu, Z. G. (2018). Tumor heterogeneity of gastric cancer: From the perspective of tumor-initiating cell. *World J. Gastroenterol*. 24 (24), 2567–2581.

[2] Hussain, A. A. M. & Lafta, R. K. (2021). Cancer Trends in Iraq 2000–2016. *Oman Med J*. 36 (1), e219.

[3] Thrift, A. P. &, El-Serag, H. B.(2020). Burden of gastric cancer. *Clin Gastroenterol.Hepatol*.18 (3), 534–542.

[4] Lauren P. (1965). The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma: an attempt at a histoclinical classification. *Acta Pathol Microbiol Scand*. 64, 31–49.

[5] Machlowska J, Baj J, Sitarz M, Maciejewski R, Sitarz, R. (2020) Gastric Cancer: Epidemiology, Risk Factors, Classification, Genomic Characteristics and Treatment Strategies. *Int J Mol Sci*.21 (11), 4012.

[6] Weskamp, G., Kratzschmar, J., Reid, M.S. & Blobel, C.P. (1996). MDC9, a widely expressed cellular disintegrin containing cytoplasmic SH3 ligand domains. *J. Cell Biol*.132,717–726.

[7] Liu, C.M., Hsieh, C.L., He, Y.C., Lo, S.J., Liang, J.A., Hsieh, T.F., Josson, S., Chung, L.W., Hung, M.C. & Sung, S.Y. (2013). In vivo targeting of ADAM9 gene expression using lentivirus-delivered shRNA suppresses prostate cancer growth by regulating REG4 dependent cell cycle progression. *PLoS One*. 8 (1), e53795.

[8] Chen, C.M., Hsieh, Y.H., Hwang, J.M., Jan, H.J., Hsieh, S.C., Lin, S.H. & Lai, C.Y. (2015). Fisetin suppresses ADAM9 expression and inhibits invasion of glioma cancer cells through increased phosphorylation of ERK1/2. *Tumour Biol*. 36, 3407–3415.

[9] Wang, Y., Balaji, V., Kaniyappan, S., Kruger, L., Irsen, S., Tepper, K., Chandupatla, R., Maetzler, W., Schneider, A., Mandelkow, E. &Mandelkow, E. (2017). The release and trans-synaptic transmission of Tau via exosomes. *Mol Neurodegener*. 12(1),5.

[10] Rinchai, D., Kewcharoenwong, C., Kessler, B., Lertmemongkolchai, G. & Chaussabel, D. (2015).Increased abundance of ADAM9 transcripts in the blood is associated with tissue damage. *F1000Res*. 4,89.

[11] Haoyuana, M.A. & Yanshub, L.I.(2020).Structure, regulatory factors and cancer-related physiological effects of ADAM9.*Cell Adh Migr*.14 (1), 165–181.

[12]Fry, J.L. & Toker, A. (2010). Secreted and membrane-bound isoforms of protease ADAM9 have opposing effects on breast cancer cell migration. *Cancer Res*. 70,8187–8198.

[13]Chou, C.W., Huang, W.K., Kuo, T.T., Liu, J.P. & Sher, Y.P.(2020). Review: An Overview of ADAM9: Structure, Activation and Regulation in Human DiseasesInt. *J. Mol. Sci*. 21, 7790.

[14]Edwards, D.R., Handsley, M.M. &Pennington, C.J. (2008).The ADAM metalloproteinases. *Mol. Asp. Med*. 29, 258–289.

[15]English, W.R., Siviter, R.J., Hansen, M. & Murphy, G. (2017). ADAM9 is present at endothelial cell-cell junctions and regulates monocyte-endothelial transmigration. *Biochem. Biophys. Res. Commun*. 493, 1057–1062.

[16]Lambrecht, B.N., Vanderkerken, M. & Hammad, H. (2018). The emerging role of ADAM metalloproteinases in immunity. *Nat. Rev. Immunol*.18,745–758.

[17]Hsia, H.E., Tushaus, J., Brummer, T., Zheng, Y., Scilabra, S.D. & Lichtenthaler, S.F. (2019). Functions of 'A disintegrin and metalloproteases (ADAMs)' in the mammalian nervous system. *Cell.Mol. Life Sci*. 76, 3055–3081.

[18]Kim, J.M., Jeung, H.C., Rha, S.Y., Yu, E.J., Kim, T.S., Shin, Y.K., Zhang, X., Park, K.H., Park, S.W., Chung, H.C. & Powis, G. (2014). The Effect of Disintegrin–Metalloproteinase ADAM9 in Gastric Cancer Progression. *Mol Cancer Ther*.13 (12), 3074-85.

[19]Wazir, U., Jiang, W. G., Sharma, A.K. & Mokbel, K. (2012). The mRNA Expression of DAP3 in Human Breast Cancer: Correlation with Clinicopathological Parameters. *ANTICANCER RESEARCH*. 32, 671-674.

[20]Stephenson, F.H. (2^{ed}). (2010). *Calculation for molecular biology and biotechnology: a guide to mathematics in laboratory*. Elsevier, Inc.

[21]Daniel, W.W. & Cross, C.L.($10th^{ed}$). (2013). *Biostatistics, a foundation for analysis in the health science*. John Wiely and sons. New York: XI+958PP.

[22]Lordick, F., Allum, W., Carneiro, F., Mitry, E., Tabernero, J., Tan, P., Van Cutsem, E., van de Velde, C., Cervantes, A. (2014). Unmet needs and challenges in gastric cancer: the way forward. *Cancer Treat Rev*. 40 (6), 692-700.

[23] Liu, Y., Zhang, Y., Zhao, Y., Gao, D., Xing, J., Liu, H. (2016). High PARP-1 expression is associated with tumor invasion and poor prognosis in gastric cancer. *Oncol Lett*.12 (5), 3825–3835.

[24]Lou, L., Wang, L., Zhang, Y., Chen, G., Lin, L., Jin, X., Huang, Y., Chen, J. (2017). Sex difference in incidence of gastric cancer: an international comparative study based on the global burden of disease study 2017. *BMJ*.10 (1), e033323.

[25]Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., *et al*. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*.136 (5), E359-386.

[26] Radkiewicz, C., Johansson, A. L. V., Dickman, P. W., Lambe, M., Edgren, G. (2017). Sex differences in cancer risk and survival: A Swedish cohort study. *EJC*.84, 130-140.

[27] Camargo, M. C., Goto, Y., Zabaleta, J., Morgan, D. R., Correa, P., Rabkin, C. S. (2012). Sex hormones, hormonal interventions, and gastric cancer risk: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 21(1), 20–38.

[28] Xie, S. H. & Lagergren, J. (2016). The Male Predominance in Esophageal Adenocarcinoma. Clinical gastroenterology and hepatology: the official clinical practice journal of the American gastroenterological association. *Clin Gastroenterol and Hepatol*.14 (3), 338–347.

[29] Piazuelo, M. B. & Correa, P. (2013). Gastric cancer: Overview. *Colomb Med*.44 (3), 192-201.

[30]Marques-Lespier, J. M., Gonzalez-Pons, M., Cruz-Correa, M. (2016).Current Perspectives on Gastric Cancer. *Gastroenterol Clin North Am*.45(3), 413–428.

[31] Cisło, M., Filip, A. A., Offerhaus, G. J. A., Ciseł, B., Rawicz-Pruszynski, K., *et al*. (2018). Distinct molecular subtypes of gastric cancer: from Laurén to molecular pathology. *Oncotarget*. 9 (27), 19427-19442.

[32] Zhou, Y., Cui, J.-G., Huang, F., Zhang, A., Li, C., *et al*. (2017). Prognostic Factors For Survival In Node-Negative Gastric Cancer Patients Who Underwent Curative Resection. *SJS*.106 (.3), 235–240.

[33] Zhang, W., He, D., Chen, D., Li, T., Chen, X., *et al*. (2016). Comparison between superficial muscularis propria and deep muscularis propria infiltration in gastric cancer patients. *Medicine (Baltimore)*.95(29), e4165.

[34] Chen, J., Cai, R., Ren, G. , Zhao, J., Li, H., *et al*. (2018). Differences in clinicopathological characteristics and computed tomography findings between signet ring cell carcinoma and non-signet ring cell carcinoma in early and advanced gastric cancer. *Cancer Med*. 7 (4), 1160–1169.

Received 15 May 2021; Accepted 20 May 2021.

[35] Lee, J. H., Park, M. S., Kim, K. W., Yu, J. S., Kim, M. J., Yang, S. W. (2006). Advanced gastric carcinoma with signet ring cell carcinoma versus non-signet ring cell carcinoma: differentiation with multidetector CT. J. *Comput. Assist. Tomogr*.30 (6), 880–884.

[36]Carl-McGrath, S., Lendeckel, U., Ebert, M., Roessner, A.& Rocken, C. (2005). The disintegrin-metalloproteinases ADAM9, ADAM12, and ADAM15 are upregulated in gastric cancer. *Int J Oncol*. 26, 17–24.

[37]Kim, J.M., Jeung, H.C., Rha, S.Y., Yu, E.J., Kim, T.S., *et al. (*2014).The effect of disintegrin-metalloproteinase ADAM9 in gastric cancer progression. *Mol Cancer Ther*. 13, 3074–3085.

[38] Grutzmann, R., Luttges, J., Sipos, B., Ammerpohl, O., Dobrowolski, F. (2004). ADAM9 expression in pancreatic cancer is associated with tumor type and is a prognostic factor in ductal adenocarcinoma. *BJC*. 90, 1053–1058.

[39]Wang, J., Zhou, Y., Fei, X., Chen, X., Yan, J., *et al.* (2017).ADAM9 functions as a promoter of gastric cancer growth which is negatively and post-transcriptionally regulated by miR-126.*ONCOLOGY REPORTS*.37 (4), 2033-2040.

[40]Seals,D. F. & Courtneidge, S. A. (2003).The ADAMs family of metalloproteases: multidomain proteins with multiple functions. *Genes and Development.*17(1), 7–30.

[41]Park, J.W., Baek, N.S., Lee, S.C., Oh, S.J., Jang, S.H., *et al*. (2014). Preclinical efficacy testing for stomach and liver cancers. *Cancer Research and Treatment*. 46, 186–193.

[42]Schfer, B., Gschwind, A., Ullrich, A. (2004). Multiple G-protein-coupled receptor signals converge on the epidermal growth factor receptor to promote migration and invasion. *Oncogene*. 23, 991–999.