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The Role of Integrin α2 (ITGA2) factor in Iraqi Females Patients with Breast Cancer

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Abstract: ***Cancer is the second leading cause of death throughout the world. Breast cancer is one of the leading mortality reasons in women from Western Countries in Iraq; breast cancer is the second reason of death After cardiovascular Diseases. Integrin are heterodimer integral membrane glycoproteins with distinct Two chainα, β, one of the receptors of adhesion molecules has an important role in metastasis. The study included (106) female sample of Iraqi people. In divided into two groups, the first group (68) patients with breast cancer, were investigated, the patients attended to (tumor unit at madienatalteb teaching hospital and Al-Amal Al-Waaniihospital in Baghdad, while second group (38) apparently healthy controls. ELISA and PCR has been used in this study. ELISA Technique has been used to determinate the serum level of integrin α 2 (an adhesion molecules) while in PCR Technique used to study the polymorphism of integrin α 2 (ITGA2) gene (C807T), the two under studying group undergo to the both technique. The serological study was carried out by using ELISA technique, while molecular study was carried out by using PCR technique, 5ml of peripheral blood had been drawn from each patients, then blood sample divided in two part (2 ml) in Gel tube for serological study, and (3ml) in EDTA tube for molecular test. By ELISA technique the results that recorded (ITGA2)a non-significant differences in patients (25.17 ± 1.15) than controls (24.20 ± 2.03). A molecular study part (PCR RFLP) employed 106 individual (68) patients and (38) healthy individual. The electrophoresis results for gene (ITGA2)at site C807T for two alleles are (T, C) showed a three genotypes (TT, CT, CC) and repeat compositions genetic (TT)that recorded a non-significant differences while the genotype (CT, CC) recorded a higher significant differences under probability P<0.01 in the patient group than control. Allele T recorded a non-significant differences in patients compared to control using the test Fisher and relying on relative factor and showed that the allele T was Etiological faction (EF).while allele C showed a non-significant difference in patients compared to controls by using test Fisher depending on relative factor showed a preventive faction (PF) of the disease. Repeat genotype TT difference is a non-significant in patient compared to control and showed style of etiological faction (EF) of disease , while genotype CT showed a significant difference under probability <0.05 in patients compared to controls and recorded style of Etiological faction (EF) of disease. Whereas a repeat genotype CC showed a non-significant difference in patients compared to controls and showed style preventive fraction (PF).***

Keywords: Breast cancer, integrin α2, C807T.

1. Introduction

Cancer is the second leading cause of death through outthe world. Breast cancer is one of the leading mortality reasons in women from Western Countries In Iraq, breast cancer is the second reason of death after cardiovascular Diseases [1]. Breast cancer is a type of cancer producing from breast tissue, usually from the inner lining of milk ducts, Or lobules that must provide the ducts with milk [2]. There were (3763) cases of breast cancer with incidence rate about (23.01) per 100, 000 female population in 2011, compared to (16.65) per 100, 00 Female population in 2008 in Iraq [1].Adhesion molecules can be divided into four Minerfamilies: the cadherin Superfamily, the selectins, the immunoglobulin superfamily and the Integrins [3-4]. The interactions of these cell adhesion with the ECM are Serious for development processes as various as the differentiation of Tissues, morphogenesis and the development of metastases [5]. The extracellular matrix (ECM) is a high dynamic building that is found in all tissues and continuously submits controlled remodelling. This process include quantitative and qualitative variation in the ECM, Mediated via particular enzymes that are responsible for ECM Degradation, such as metalloproteinases. The ECM interacts with cells to Organize different functions, involve proliferation, migration and Differentiation [6], otherwise ECM as well as participate in a critical Way to the progress of cancer cell invasion and metastasis [7].Integrins are the main and best-describe transmembrane receptorsthat Mediate dynamic interactions among the extracellular matrix and the Actin cytoskeleton through cell motility. Integrins are the main and most Recognized ell surface receptors of many Extracellular matrix (ECM) Proteins for example laminin, fibronectin, collagen IV and vitronectin. Integrins are formation of non-covalent, heterodimeric complexes of an α and β subunit [8]. Several members of the integrin family, for example α5β1, α8β1, αIIbβ3, αVβ3, αVβ5, αVβ6, and αVβ8 characters an Arg-Gly-Asp (RGD) motif during their ligands, which contain FN, Fibrinogen, vitronectin, von Willebrand factor, and different other large glycoproteins [9].

Integrins constitute the mediators between ECM and the actin Cytoskeleton with focal adhesion locations representing the areas of Signal transduction, wound healing, differentiation, controlling Proliferation, survival migration, tumorigenesis, etc. *[*10] Integrin are heterodimeric integral membrane glycoproteins with distinct Two chain. They are found on a wide differ of cell kinds including, T cell (Th, NKTcells), NK cells, fibroblast and platelets, integrins are Involved Into cell adhesion and also participate in cell surface Intermediated signaling [11]. Both the α and β subunits are Transmembrane glycoproteins. As the cytoplasmic tails of integrins are Devoid of enzymatic features, they transduce signals via linking with Adaptor proteins that bind the integrin to the cytoskeleton, cytoplasmic Kinases, and transmembrane growth factor receptors [12]. It has been suppose that bone metastasis obtain from in progress prostate Cancer process is described

via the integrin-mediated interaction of Metastatic cancer cells and bone microenvironment [13].
Expression integrin alpha 2 beta 1suppressor of metastatic breast tumor Development addition integrin α2β1and play role important in cancer Migration and invasion in resent study have observed the α2β1 integrins is organized of cancer metastasis either via enhancing or inhibiting the Dissemination process of cancer cells [14]. α2β1 integrin functions as a Collagen receptor on platelets and fibroblasts and as both a collagen and laminin receptor on endothelial cells and most epithelial cell kinds [5-6]

Metastasis is one of the most important troubles cancering mortality in Cancer patients [15-17] it is a multiple steps, complex process composed Of a cascade of inter linked event including intravasation, adhesion to the Vessel wall, extravasation, infiltration, and proliferation in to target Tissue [16]. Many of these steps include integrins which recognized to Exist in distinct activation states, which show various affinities for ligand Generally, integrin activation controls cell adhesion [17] such control is Specially important in the vasculature where dynamic supply physically Opposes cell connection. Complexity from metastatic disease are the Primary reason of death in breast cancer, metastasis to bone, Lungs Liver and brain involves dissemination of t cancer cells by the blood Stream [15]. Functional polymorphisms intergrin genes ITGA2 C807T influence development and progression of breast cancer rick and role it metastasis and ITGA2 C807T have been linked with cell –surface intensity of integrin α2β1 [14]. Thus one of the main tasks of oncology research is the growth if inhibitors selectively targeting extracellular molecules that Constitute a crucial inter connection of multiple metastasis pathways. The aim of this study is to shied a light on integrin α2 which it may represent Therapeutic target against metastasis.

2. Materials and Methods

2.1. Samples Collection

The study was approved by the medical ethics committee of Iraqi ministry of health and was done on 68 Iraqi women (patients with breast cancer)were investigated, the patients attended to (tumor unit at Medical city teaching hospital and Al-Amal Al-Waanii hospital in Baghdad, for diagnosis and treatment during the period October/2016-January /2017, the disease was diagnosed by consultant medical staff at the hospital by using diagnostic criteria the diagnostic was made according to clinical, mamografical, histological finding, there were 68 patients (females) their age rang (25-55), and 38 apparently healthy controls (female)matched patients age (20-48 years) and ethnicity (Iraqi) were enrolled in the study. Patients were early detected (without treatment with chemotherapy or any drug. The serological study was carried out by using ELISA technique, while molecular study was carried out by using PCR technique, 5ml of peripheral blood had been drawn from each patients, then blood sample divided in two part (2 ml) in Gel tube for serological study and (3ml) in EDTA tube for molecular study. Carried out by using Eliza kite Human Integrin Alpha-2 were purchased from CUSABIO**,** CHINA storied in -4◦C

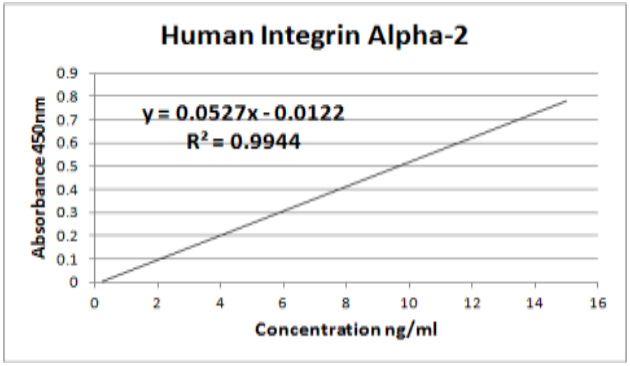

Figure 1. Standard curve of Human integrin Alpha -2

2.2. DNA Extraction

Was carried out by procedure supply with DNA Extraction kit purchase from Geneaid Taiwan.

2.3. Enzyme

Enzyme Hinf I was purchased from Bioneer, Korea and stored -20◦C [18]

2.4. Primers Used for the Study

Primers were purchased from Bioneer, Kore.

Table 1: Sequence of primer used in this study **(**35)

Primer	Sequence 5 3
Forward (F)	*5gatttaactttcccagctgccttc3*
Reverse (R)	*5atggtggggacctcacaaacagatt3*

Table 2: PCR reaction Components for amplification

Component	*Quantity (µl)*
Forward primer	*2*
Reverse primer	*2*
DNA template	*4*
D.W	*12*
Master mix	*5*
Final volume	*25*

Table 3: PCR amplification programm ITGA2C807 gene

Steps	Temperature c	Time	No. of cycles
Initial denaturation	95	3 minute	1
Denaturation	95	30 sec	35
Annealing	60	45 sec	
Extension	72	30 sec	
Final extension	72	5 minute	1

2.5. iserohportcelEleGesoragA

1.5% agarose gel containing ethidium bromide idetalos DAN sawdezilausivno10mg/ml) in 1xTAE buffer at 70 volts for half hour and 100 volts for one hour (running time one Hour)

Table 4: RFLP Programs

Component	*Component of sample (µl)*
PCR product	10
Hinf 1 Enzyme	1
BSA	2
Buffer	2
D.w	5
Total volume	20

Incubate for 3hr at 37°C.

2.6 Restriction fragment Length polymorphism (Hinf I)

Restriction digestion of PCR product was carried out with a Total volume of 20µl consisting 10µl from pcr product, 5µl of D.W, 2µl Buffer, , 2µl BSA and 1µl of enzyme Hinf 1.Samples were incubated at37◦C for 3h. RFLP pattern was visualized on 2% agarose consisting ethidium bromide 10mg/ml) in 1x TAE buffer at 100 volts for 90 min.

2.7 Statistical Analysis

In order to analyze the result, the Statistical Analysis System- SAS (2012) program was used to study the effect of different factor in study parameters. Least significant difference –LSD test, t test were used to significant compare between means in this study. Estimate of correlation coefficient between difference variables**.** Estimate of correlation coefficient between difference variables. Alleles and genotypes were presented as percentage frequencies, and significant differences between their distributions in breast cancer patients and controls were assessed by two-tailed Fisher's exact probability (P). In addition, relative risk (RR), etiological fraction (EF) and preventive fraction (PF) were also estimated to define the association between alleles and genotypes with the disease. The RR value can range from less than one (negative association) to more than one (positive association). If the association was positive, the EF was calculated, while if it was negative, the PF was given. (**45.46)**.

3. Results and Discussion

3.1 General characteristics of patients

As shown in Table 5The result showed that there were two histological subtype, Ductal and Lobular (98.5%-1.47%) respectively. The age median for all patients groups (<35, 35-45 and > 45) percentage was (23.5%, 44.1% and 32.3 %) respectively. The study was included four stages II B, II A, III C, III A The number patient for each stage (n4669.6%, 9 13, 2%, 7 10.2%, 6 8.8%) respectively, grading recorded (5.8 %, 67.6 %, 26, 4%) Grad I, II, III Respectively. and TNM stage (7.3%, 57.3%, 35.2%) T0, T2, T3 respectively. (60.2%, 22.0%, 7.3%, 4.4%, 5.8%) N0, N1, N2, N3, N4 Respectively. M0 (100).

Table 5: Clinical and pathological characteristics of breast cancer patient's diagnosis

citsiretcarahC	**stneitaprecnactsaerB** (No=68) (%)
epytbuscigolotsiH	
latcuD	67 (98.5)
raluboL	1 (1.47)
naidemegA	68 (25-55)
35nahtsseL	16 (23.5)
35-45	30 (44.1)
45nahteroM	22 (32.3)
Segat	
II B	46 (69.6)
II A	9 (13.2)
III C	7 (10.2)
III A	6 (8.8)
edargcigolotsiH	
edarG I	4 (5.8)
edarG II	46 (67.6)
edarG III	18 (26.4)
romuTetis	
tfeL	34 (50)
thgir	34 (50)
egats MNT	
0T	5 (7.3)
2T	39 (57.3)
3T	24 (35.2)
0N	41 (60.2)
1N	15 (22.0)
2N	5 (7.3)
3N	3 (4.4)
4N	4 (5.8)
0M	68 (100)

TNM (Tumor Node Metastasis)

3.2 Histological subtype discussion

The most invasive tumor kind, invasive ductal cancer was the most spread histological tumor kind. The percentage was in patient 98.5% for 67 cases, while the subtype invasive Lobular cancer was founded with percentage ratio in patient 1.47% as shown inTable-5.the present study showed that the histological tumor type invasive ductal cancer was the common histological kind which is agree with other study [18-23].while Lobular invasive carcinoma was the second most spread histological type the percentage ratio inpatient 1.47%.while the histological tumor type Lobular carcinoma in present study is a lower compared with other study (15.3%) morocco, Egypt (9%), Iraq (39%), Saud Arabia (3%) [18, 24, 25, 26].

3.3 Stages discussion

Staging is the process of finding out how widespread the cancer is when it is found, the stage is the most important factor in deciding how to treat the cancer and determining how successful treatment might be [49] the stage it allows the preliminary evaluation of disease. according to AJCC/TNM (tumor node metastasis)classification system there were four stages, patient which enrolled in this study were divided into four groups of disease stages as described in Table-5, these groups included III B, II A, III C, IIIA the percentage of patients in each group for each stage was (69.6%, 13.2%, 10.2%, 8.8) respectively, this result as compared with other Iraqi study which had a number of patients 721, 76%of them were in stage I. (45.2) in stage II

while (5.7%) were in stage Iv [27, 28] identical finding has been observed in Iraq by [29, 30] which recorded (6%, 48%, 33%, 13%) for stage I, II, III, IV respectively. While in other countries recorded as much higher frequencies stage I, II, for example Sweden and Florence, in these countries a reduction in the frequency of advanced carcinomas (stage II B and II A and more) which it observed [31]. And other recent studies in Iraqi showed that younger patients have demonstrated advanced stage IV risk more with the progression of breast cancer [27, 28].

3.4 Grades discussion

Histological grading system also has different types that an important in breast cancer progress [32] it has been recorded that the average of survival for 10 year in grade I tumors patient was about 80% then this percentage will lower to 45% in grade III tumors [33]. There were two grads, patients which enrolled in this study that there were divided into four groups as described in Table-5, these groups include I and II with a percentage about (5.8%, 67.6%) respectively for each group and this finding was agree with [27, 28] which recorded a percentage for I, II, III grade patients (3.5%, 56.6%, 39.9%) so we may conclude that grad II patients has a high proportion (68%).grade III patients has a percentage 26% which agree with [34] which recorded a percentage of Grad II III (67 %, 33%) respectively. but our finding was disagree with [18] who recorded a percentage for grade III 18%. In other studies has founded were a percentage Grade I 11%, Grade II (58 %), Grad III 31% [30], TNM stag e according to AJCC /TNM classification system [35, 36].

3.5 Integrin alpha 2 (ITGA2 ng/ml) level in serum and Genotyping

In present study as shown in table 2. The result of The integrin alpha -2 level serum showed a non-significantly increasing in patients group (25.17 ± 1.15) compared to control group (24.20 ± 2.03) by using a statistical testing (t test) under probability P<0.05.as shown in Table 6.

Table 6: Statistical analysis for ITGA2 ng/ml Level in serum of patients comparing to control

The group	No.	Mean ± SE of ITGA2
Patients	64	25.17 ± 1.15
Control	24	24.20 ± 2.03
t-Test	---	4.686 NS
P-value	---	0.6798

NS: Non-Significant

Integrins are formation of non-covalent, heterodimeric complexes of an α and β subunit [42] α2β1 integrin functions as a Collagen receptor on platelets and fibroblasts and as both a collagen and laminin receptor on endothelial cells and most epithelial cell kinds [5-6] The integrin alpha 2 beta 1 play an important role in cell migration, cell invasion in to collagen and integrin have also important role to signal transduction, integrin alpha 2 beta 1 has Addison Capacity of boosting cell proliferation depending on the kind and physical state of collagenous matrix [47].

Although there were a lot of study which asses the concentration of integrin alpha 2 ITGA2 as protein in tissues but this is the first time which asses its concentration in the serum (locally and universally). Our result showed that there are lower serum level ITGA2 in both patients and control and there were a non-significant difference between them according to the p value under prop < 0.05 as shown in Table 6, The result was disagree with [37] who said the lower ITGA2 protein level was recorded in breast cancer compared to close non-cancerous breast tissues p>0.001. In addition the previous studies recorded that phosphates of regenerating liver-3 repressed ITGA2 expression in ovarian cancer cell at the transcription level [38] and other study COX2 increased active ITGA2 expression during the ep1/plc/pkca2 c-sre/ nf.kb signal transduction cells [39]. Cell extracellular matrix (ECM) play important role through embryonic growth, wound healing [40] the assertion that epigenetic modification of the ITGA2 enhancer is a mechanism via which ITGA2 expression is organized [41].

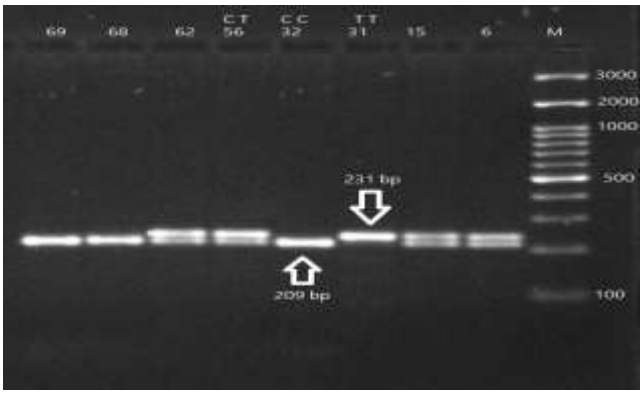

Figure 2: Genotype polymorphism of integrin α2C807T gene which using PCR-RFLP illustrate genotype TT in the tasks (31) just band 231bp, genotype CT in the tasks (6, 15, 56, 62)band 231bp with band 209bp and genotype CC (32, 68, 69) band 209 bp.

The present study showed the length of the extracted DNA (231bp) after digestion by the restriction enzyme and after Electrophoresis was long (209 bp, 231 bp) nitrogenous base to the genotype (CT) (heterozygotes) while it was long (209 bp) nitrogenous base to the genotype (CC) (homozygotes), the genotype (TT) (homozygotes) was long (231 bp) nitrogenous base.

Table (7) About statistical analysis shows that there are anon significant differences in patients compared to control by using Fisher's Exact Probability, frequency of genotype TT recorded the relative risk (1.30) value can range more than one (positive association) and show as etiological or preventive fraction of infection diseases its risk (0.034) it showed anon-significant differences (0.920)according to fishers exact probability while confidence intervals value among (0.46-2.33)under percentage 95%.The genotype CT recorded the relative risk (2.83) and show as etiological or preventive fraction of infection diseases its risk (0.647) it showed a significant differences (0.051) according to fishers exact probability while confidence intervals value between (0.91-10.47). in statistical analysis by using Fisher s test genotype CC showed the relative risk (0.51) and showed as etiological or preventive fraction of infection diseases its risk (0.485) and shown a non-significant differences (0.114) according to fisher s probability and confidence intervals value among (0.22-1.19) under percentage 95%. The allele

frequency T showed the Relative Risk more than one (positive association) 1.39 as well as Etiological fraction, Fisher s Exact probability and confidence intervals under percentage 95% (0.278, 0.314, 0.76-2.53) respectively and fisher s test recorded anon significant different. While the allele frequency C showed the Relative Risk less than one (negative association) 0.72 with preventive fraction, Fisher s Exact probability and confidence intervals under percentage 95% (0.728, 0.314, 0.39-0.72) respectively. Soallele T may consider as a causes to increase the incidence of disease while allele C may consider as a protective allele.

Table 7: Statistical evaluations of C807T gene

C 807 T Genotype and Allele	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
TT	1.30	0.034	0.920 NS	0.46-2.33
CT	2.83	0.647	0.051 *	0.91-10.47
CC	0.51	0.485	0.114 NS	0.22-1.19
T	1.39	0.278	0.314 NS	0.76-2.53
C	0.72	0.728	0.314 NS	0.39-0.72

*** (P<0.05), NS: Non-Significant**

Our current study agreement with [14] which showed that genotype TT recorded the relative Risk (1.36) more than one (positive association), and confidence intervals (0.93-2.00) under percentage 95%, while our study disagreement with [14] who said that the genotype CT of the relative risk (0.89) less than one (negative association) and confidence intervals (0.67-1.18), as well as our current study disagreement with [14] which showed that genotype recorded the relative risk (1).This study agreement with [43] which that recorded the CT genotype was linked with an increased danger of gastric cancer (adjusted Rr= 1.54, 95% CI = 1.10-2.18, *P* = 0.013).So we may can suggest the CT genotype was linked with an increased risk of breast cancer.

The result of present study for the genotype of integrin α C807C showed that the genotype TT has a high percentage (45.59%) in patient compared with all genotype (CT, CC) (25.00%, 29.41%) Respectively, Chi-square for (TT)genotype showed a non-significant difference (0.538) in patient compared to control, while the genotype (CT, CC) showed a high significant difference (6.198, 6.263) Respectively compared with control under probability (P<0.01), as shown in Table 8.

Table 8: Analysis of the genotyping distribution for integrinα2 genei C807T in patients compared to control.

Genotype	Patients	Control	Chi-square	P-value
TT	31 (45.59%)	17 (44.74%)	0.538 NS	0.839
CT	17 (25.00%)	4 (10.52%)	6.198 **	0.0118
CC	20 (29.41%)	17 (44.74%)	6.263 **	0.0115
Total	68	38	--	--

** (P<0.01) high significant, NS: Non-Significant.

Present study illustrated that the genotyping distribution for integrin α2C807T gene was as Genotype CC showed higher percentage (29.41%) for 20 from patients compared with genotype CT which recorded lowest percentage (25.00%) in patient while in control the genotypes (TT, CC) recorded an equal percentage (44.745) for both of them, while CT genotype recorded lowest percentage ratio (10.52%) for 4 from control, the chi- square recorded a non-significant difference for Genotype in patient compared with control while genotype CC, CT recorded a highest significant difference for patient compared (P < 0.01) as shown Table8, Figure2.

Present study disagreement with the previous study, which showed that integrin α2 C807T gene was as follow, the genotype CC showed medium percentage (38.6%) in patient compared with other genotype CT, TT (52.5-8.9%) respectively. The genotype CC, CT recorded the percentage (43.6%) for control compared with other genotype, TT recorded a lowest percentage (12.8%), who said the integrin α2 C807T genotypes not association with the main prognostic different of breast cancer patient [35].Another previous study [42] recorded that ITGA2 gene C807T polymorphism was linked with different diseases, including stroke, retinal vein obstruction, acute coronary syndrome, colorectal cancer. Other study showed integrin α2encode the α subunit of integrin α2β1 that which plays significant role for normal mammary epithelium in breast cancer tissue [35].Moreover another article showed that ITGA2 has a role in progression of gastric cancer, its appear that the polymorphism perhaps linked with danger of gastric cancer [43]. Recent study demonstrated that functional polymorphisms intergrin ITGA2 gene C807T influence development and progression of breast cancer and has a role in metastasis and it have been linked with cell –surface intensity of integrin α2β1 [14] while there was an opposite opinion which mention that integrin polymorphisms not linked with relapse free and survival in colorectal cancer patients [44].

The present study Showed comparison of integrin alpha-2 (ITGA2 ng/ml) level according to the genotype in control and patients. The genotype TT showed non-significant (23.79 ± 4.78)in patient compared to control (27.08 ± 4.78).as well as genotype CT recorded a non-significantly increased (25.56 ± 4.96) in patient compared to control (20.80 ± 3.61).the same for genotype CC showed to a non-significant increased (26.57 ± 5.56) in patients Compared to control (20.14 ± 3.93). as shown in **Table.9**

Table 9: The comparison level integrin alpha-2 (ITGA2 ng/ml) according to type of genotype in patients and control

AGTI2	tneitaP naeM±ES	Control naeM±ES	t-test
TT	23.79 ± 4.78	27.08 ± 4.78	5.618 NS
TC	25.56 ± 4.96	20.80 ± 3.61	6.834 NS
CC	26.57 ± 5.56	20.14 ± 3.93	7.052 NS

NS, non-significant

In present study the result of correlation integrin alpha 2 (ITGA2) with histological study showed anon significantly differences, the result of the tumor histological type, stage, grade, age groups were, 25.04±7.67 +++ , 34.12±0.00 +, 29.76±7.22 +++, 23.82±4.96 +, 27.54±6.21++, 26.03±2.96+++, 22.50±2.17 +, 26.24±3.84 +++, 26.22±3.75 +++, 33.17±5.88+++, 22.68±4.07+, 27.29±8.64++) respectively. Our study showed that ITGA2 Protein level in serum was no associated with all data as shown in **Table 10.**

Table 10: Association integrin alpha-2 (ITGA2 ng/ml) with clinical pathology

Characteristic	NO	naeM±ES	Value DSL
epytbuscigolitsiH			
latcuD	67	25.04±7.67 (+++)	17.348
raluboL	1	34.12±0.00 (+)	NS
edarG			
1	4	29.76±7.22 (+++)	
2	46	23.82±4.96 (+)	8.048 NS
3	18	27.54±6.21 (++)	
egats			
BII	46	26.03±2.96 (+++)	9.975 NS
AII	9	22.50±2.17 (+)	
CIII	7	26.24±3.84 (+++)	
AIII	6	26.22±3.75 (+++)	
25-50)egA(			
35 nahtsseL	16	33.17±5.88 (+++)	14.478
35-45 45nahteroM	30	22.68±4.07 (+)	NS
	22	27.29±8.64 (++)	

NS :Non-significant
Low (+), medium (++), high (+++

This result agree with [37] which that the ITGA2 protein level was not related to TNM stage, histological grade, age group.

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