

Detection of CD3 & CD19 of T & B Lymphocytes Respectively In Gastric Carcinoma

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Abstract

Summary

Background: Gastric carcinoma is a common tumor in our country & world wide. It has been classified by Lauren (1965) into intestinal, diffuse & heterogeneous combination types. Lymphocytes express surface molecules, thus a systemic nomenclature has been developed for these surface markers (molecules) - the Cluster of Differentiation (CD) system numbered as CD1, CD2, etc. These markers can be identified by specific monoclonal antibodies. The aim of the study to detect tumor infiltrating lymphocytes (TILs), T & B lymphocytes in gastric carcinoma & to assess the correlation of intensity of these cells with the tumor size, site, lymph node involvement, grading & staging of the tumor.

Materials & Methods: Thirty four cases (gastrectomy specimens) were included in this retrospective study. T & B lymphocytes had been detected by using CD3 & CD19 markers respectively by immunofluorescent method & these were correlated with the various parameters of gastric cancer.

Results & Conclusions: Most patients were old males. Our results showed that most of carcinomas were located in the pylorus & the antrum. Taking in consideration the largest diameter of tumor mass, all the cases show tumor size ≥ 2 cm, most of them present with nodal stage (N1). Half of the cases were poorly differentiated. Most of the cases presented with stage III. CD3 & CD19 expression was correlated with the LNs involvement. CD3 was more expressed in the well differentiated & with the fewer LNs metastasizing tumors; that explain the antitumor effect of cytotoxic T-lymphocytes as a defense mechanism. There were insignificant correlations between the expression of CD3 & CD19

& the above clinicopathological parameters.

Key word:

: CD3 and Cd19 immunofluorescent markers in gastric carcinoma

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Introduction:

Gastric carcinoma is a common tumor in our country & world wide.¹⁻² It has been classified by Lauren (1965) into intestinal, diffuse & heterogeneous combination types .³Lymphocytes express surface molecules. A systemic nomenclature has been developed for these surface markers (molecules) - the Cluster of Differentiation (CD) system numbered as CD1, CD2, etc. These markers can be identified by specific monoclonal antibodies. The aim of the study to detect tumor infiltrating lymphocytes (TILs), T & B lymphocytes in gastric carcinoma & to assess the correlation of intensity of these cells with the tumor size, site, lymph node involvement, grading & staging.

Materials & Methods:

A retrospective study on 34 cases of gastric carcinoma (GC) diagnosed at histopathological laboratory of gastroenterology and hepatology teaching hospital in Medical City during the period between (January 2003 April 2008). The patients were diagnosed by examining the H & E stained slides of the gastrectomy specimens. The cases were analyzed according to patient's age, sex, site, size, grade and stage of tumor (according to the TNM staging system)⁴and lymph node involvement . Thirty four cases were submitted to immunofluorescent (IF) study. This formalin fixed, paraffin embedded tissue slides were stained for Pan B-cell antigen (CD19) & Pan T-cell antigen (CD3) markers.

Interpretation and Evaluation of Immunological stains : The results of immunofluorescent assays should show a number of desired color spots with color that denotes specificity .⁵ In dealing with FITC/CD3, RPE/CD19 the presence of a green or red color reaction product respectively at the site of the target antigen is indicative of positive reactivity. Normal lymph node tissue section stained as positive control while negative control was done by omitting primary antibody addition step. Diffuse staining appearance was regarded as non specific. Sporadic non specific colored staining of connective tissue may also be observed in sections of formalin fixed tissues. CD3 & CD19 antigens were regarded reactive if the lymphocyte cells showed staining. Statistical analysis was computer assisted by using SPSS version 11 (statistical package for social sciences). Chi square test & ANOVA test had been used. Standard deviation (SD), correlation coefficient (R) & the mean have been calculated. P-value less than 0.05 were considered as significant.

Results:

The mean age of patients was 50.38 ± 12.62 years with a range of 23-78 years. Two patients were 20-29 years; three patients were 30-39 years age groups. Twenty out of thirty four cases (58.8%) were of the age group 40-59, five cases were 60-69 years & four patients were 70-79 years. Twenty four patients were male; whereas only 10 patients were female. The male to female ratio was 2.4:1.

In a descending order; our results showed that 44.11% of carcinomas were located in the pylorus, 41.17% in the antrum, 5.88 % in the cardia, 5.88% was involving nearly whole stomach & 2.94% in the fundus.

Distribution of Cases According to Tumor Size:

Taking in consideration the largest diameter of tumor mass; all the cases show tumor size ≥ 2 cm, mean size of carcinomas was 6.95 ± 4.7 cm with a range of 2-6 cm, (fig.1).

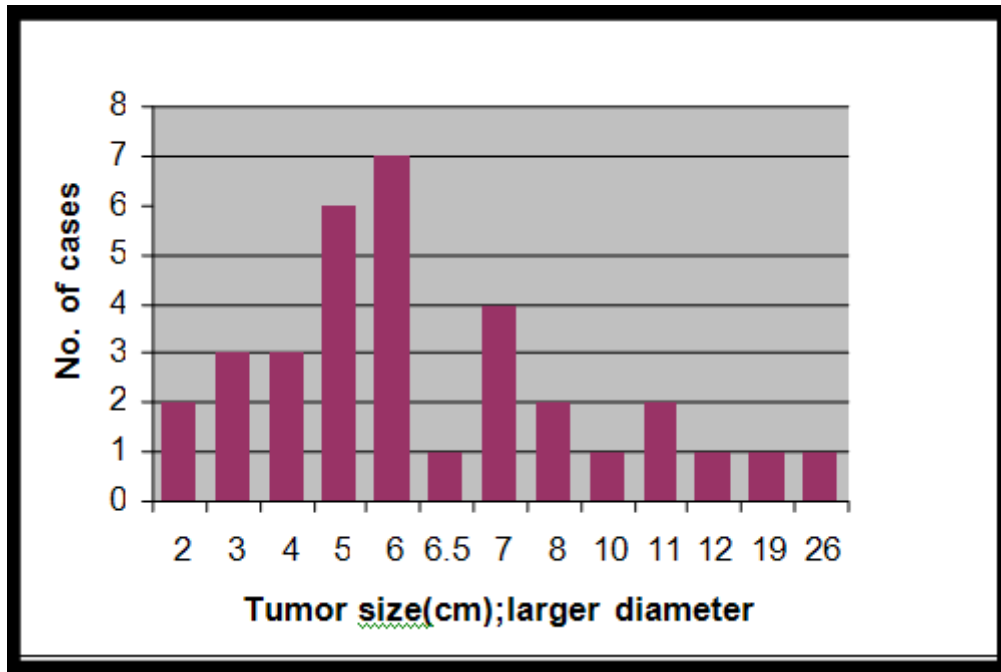


Fig.1: Histogram represents the distribution of cases according to tumor size.

Distribution of Cases According to LN Status:

Twenty four patients (71%) have positive lymph node metastasis; and 10 patients (29%) have not. In fifteen cases the carcinoma was metastasizing to 1-6 lymph node (N1), while eight cases had 7-15 lymph nodes metastasis (N2) & in one case more than fifteen nodes were involved (N3). Distribution of Cases According to the Grade of the Tumor:

Half of the cases (17 cases: 50%) were poorly differentiated, while

slightly less than half (16 cases: 47%) were moderately differentiated, and one case had a well differentiated tumor.

Distribution of Cases According to Stage:

Most of the patients presented with stage III (24 cases : 71%) while the remaining ten (29%) cases had stage II .Thirty three patients 97.05% presented with T3 stage, (fig.2).

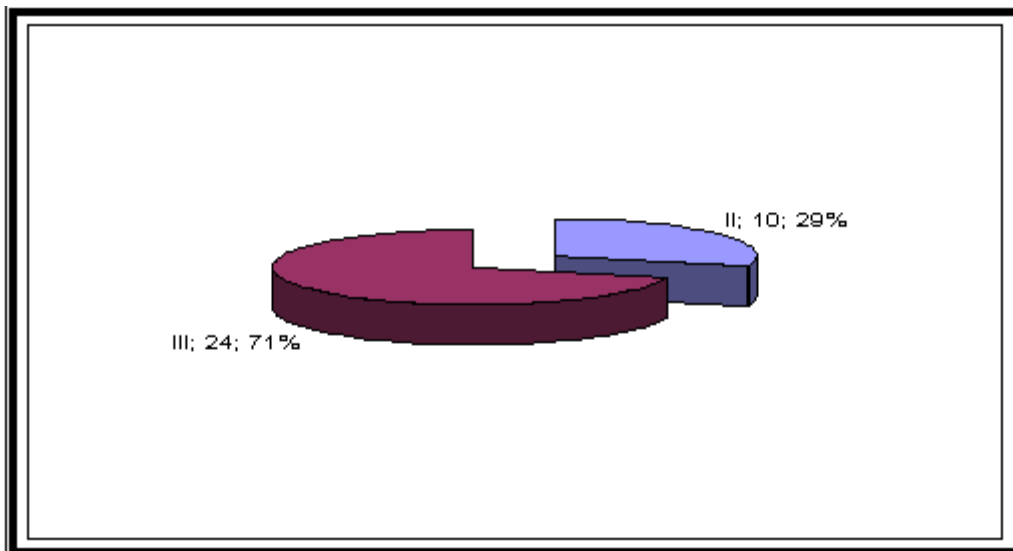


Fig.2: Pie chart represents the distribution of cases according to the stage

Distribution of cases according to Histopathological type:

Fourteen case (41%) were of intestinal type & the other twenty cases (59%) were of diffuse type (fig.3)

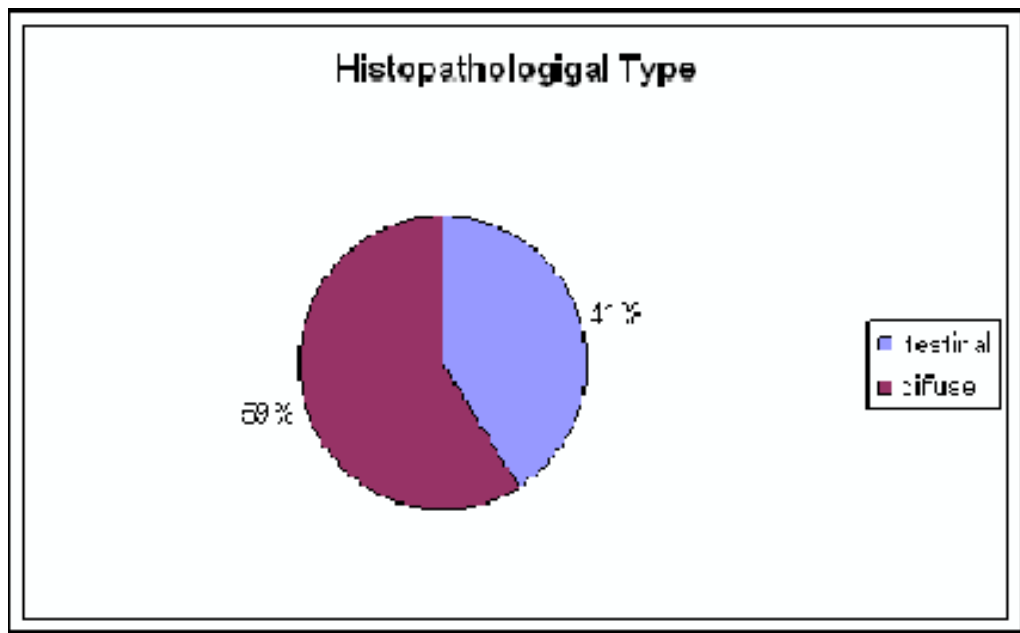


Fig 3: Pie chart represents the distribution of cases according to histopathological

Relation of CD3 & CD19 with gender:

CD3 expression (fluorescent spots /high power field) was expressed more in males

whereas CD19 expression was more in females, although the correlation was not significant (fig.4).

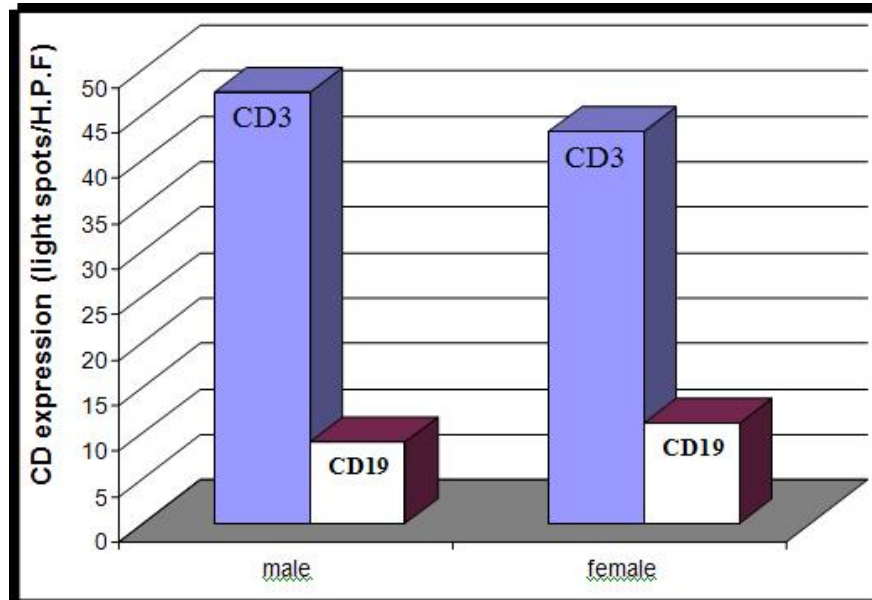


Fig.4: Histogram represents the relation between CD expression and sex.

Relation between CD3, CD19 expression and age :

Our results showed that there was a negative correlation between patient's age and

CD3 & CD19 expression (fluorescent spots/H.P.F.) but it is not significant CD3: $r=-0.133$, $p= 0.551$; CD19: $r=-0.277$, $p=0.139$, (fig.5 and fig.6).

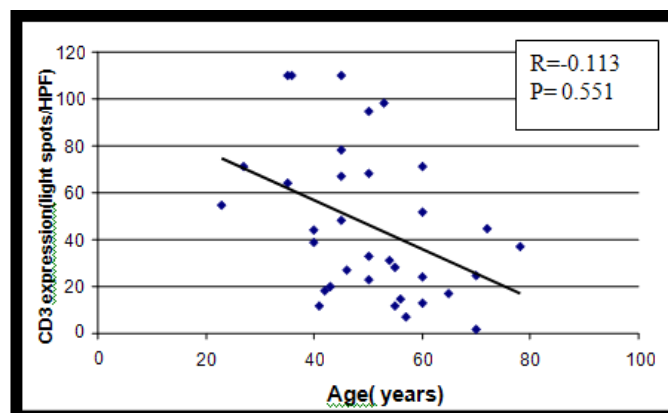


Fig.5: Scatter diagram represents the relation between the age of the patients and Cd3.

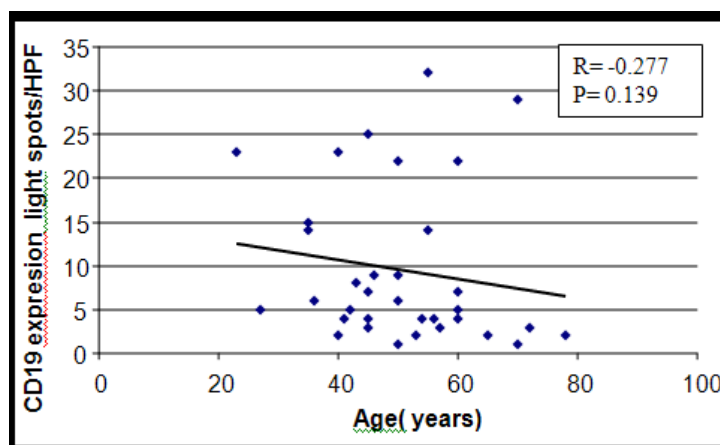


Fig.6: Scatter diagram represents the relation between the age of the Patients and CD19.

Correlation of CD expression with the site of tumor:

CD3 marker was expressed mostly in the cardia tumors, while CD19 marker was expressed mostly in the fundic tumors, but the correlation was not significant ; P-value = 0.171 & 0.665 respectively.

Relation of CD3 and CD19 expression with LN status:

Cases with LN involvement expressed CD3 (green light spots) less than that without LN involvement, while the inverse was true with CD19 expression (red light spots); these markers were expressed more in cases with LNs involvement; However the correlation was not significant, P value = 0.49 & 0.52 respectively.

CD3 expression was negatively related to the number of metastasized LNs (R-value: correlation coefficient= -0.102), whereas CD19 expression positively related to the number of LNs (R= 0.166). The correlation was not significant in both (P-value = 0.565 & P=0.349 respectively).

Relation of CD expression with the grade of the tumor:

CD3 marker expression was more in the well differentiated tumor, while Cd19 expression was prominent in the moderately differentiated carcinomas. However P value was not significant P=0.998 & P=0.528 respectively.

Correlation between CD3 and CD19 expression with tumor stage:

Regarding tumor stage, CD3 expression was more prominent with stage II , whereas CD19 expression was more in stage III tumors.

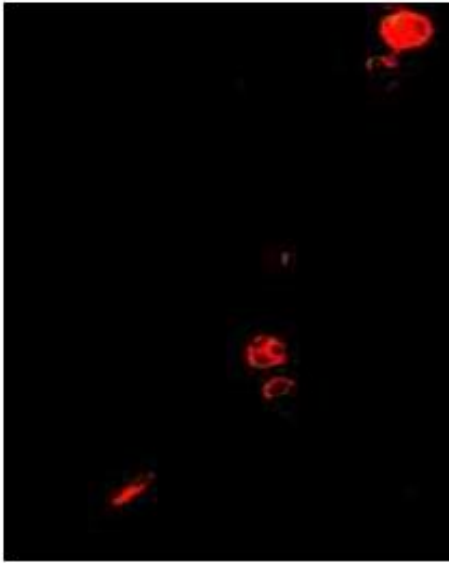


Fig.7: Tissue section shows red light spots representing CD19 antigen. This case was a 43 years old male had a pyloric, 5cm size tumor with N1 stage (X40).

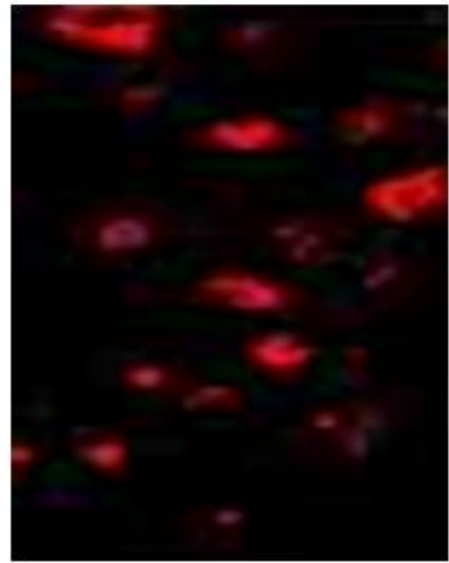


Fig.8: A high power view demonstrating red light spots of CD19 antigen. This case was a 35 years old male had a moderately differentiated and stage III tumor (X100).

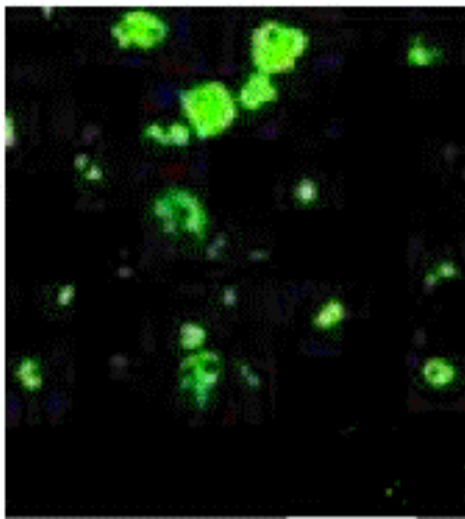


Fig.9: Tissue section shows green light spots representing CD3 marker. This patient was a 53 years old male, had a poorly differentiated and stage II tumor (X40).

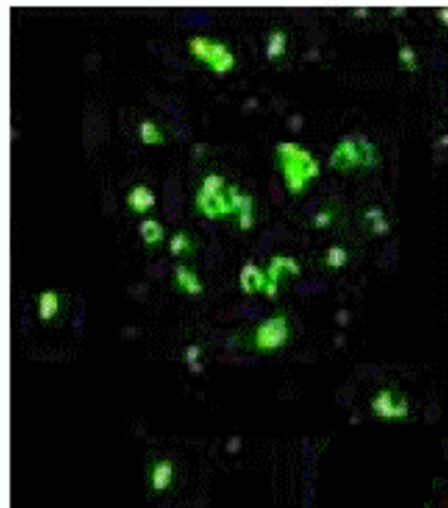


Fig.10: CD3 green light spots in 42 years old female had antrally located and 7cm size tumor (X40).

Discussion:

Gastric carcinoma (GC) is one of the most common malignancies world wide.¹ It occupied the 8th order in the list top of the ten cancers in Iraq.² Several clinical, biological and genetic parameters have been used to assess the prognosis and to help the clinician in optimizing therapies for GC patients.⁶ Studies indicated that the most important prognostic variable is the tumor stage. However, patients who are apparently at the same pathological stage often have different outcomes.⁶ Recent efforts have focused on identifying new prognostic factors that predict the clinical outcome of GC patients, with the goal of providing a rational approach or planning a specific therapy.⁶ In most parts of the world, adenocarcinoma of the stomach is primarily a disease of older individuals and is rare under the age of 40 years. Most patients are over 50 years of age.⁷ In the present study, the peak in the age of patients was closely near to the that value & near to Al-Sayegh Z.E., Saeed S. A., Kassir Z. & Vida Milašienė et al.^{8,9,10,11}

where Al-Sayegh Z.E., and Saed S.A. studies demonstrated the peak of age incidence 50-60 years; While the data reported by Kassir Z. demonstrated that (96.08%) of cases were above 40 years.

Gastric carcinoma exhibits a male to female ratio of about 2:1.¹¹ The ratio in the present study was (2.4:1), which was relatively near to the text value: However, it was different from Al-Saygh Z.E. & Saeed S.A. studies that showed a smaller male to female ratio (1.4:1).

^{8,9} Carcinoma involving the distal stomach is seen much less frequently in persons born

in North America or Western Europe than it is in persons born in Third World countries.¹²

The location of gastric carcinoma within the stomach was as follows: (44.11%) were located in the pylorus, (41.17%) in the antrum, (5.88 %) in the cardia, (5.88%) in nearly whole stomach, and (2.94%) in the fundus. These values were different from Al-Saygh Z.E. & Saeed S.A. works. Al-Saygh Z.E. Data presented that most of (66.7%) tumors located in the body, while Saeed S.A. data presented with a large number (44%) of tumors located in the gastroesophageal junction.^{8,9} Early carcinomas are 2.0 cm or less in diameter, although cases as large as 8.0cm have been described.^{13,14} In our study; all the cases showed tumor size = 2cm, this data was different from Al-Sayegh Z.E. work, who demonstrated 45.6% of cases were equal or more than 5cm in diameter.⁸

In the present study, most (71%) of the cases have positive lymph node metastasis; This is near to the data reported by Al-Sayegh Z. E. that demonstrated 78.9% of tumors have positive lymph node metastasis.⁸ In our study most of the patients presented with nodal stage (N1), this was near to the data reported by Kassir Z., in which all gastrectomy surgical specimens were associated with regional LNs involvement.¹⁰

Regarding the tumor grade half of the cases were poorly differentiated, (47%) of tumors were moderately differentiated & (3%) of carcinomas were well differentiated. This was near to Al-Sayegh Z.E. work in which 59.7% of the tumors were poorly differentiated,

36.8% of the tumors were moderately differentiated and the remaining 3.5% were well differentiated.⁸ Most of the cases in our study presented with stage III, while the other cases have stage II. These values were closely near to the data of Al-Sayegh Z. E. study in which 70.02% of the tumors were in stage III, while 15.8% in stage II.⁸

There was a negative correlation between age and CD3, CD19 expression, but it was not significant, this is near to Hong et al. study who demonstrated that the changes in lymphocyte subsets when analyzed with regard to age and sex, no significant relation has been observed between these factors and subsets.

¹⁵CD3 expression was more in male whereas CD19 expression was less. This correlation was not significant, and it was relatively similar to Hong et al results.

¹⁵Cases with LN involvement (positive LN) expressed CD3 less than that of negative LN involvement. These data support the notion that local triggering of cellular immune responses in GC prevents lymph node metastasis formation.

¹⁶CD3 expression was inversely related to the number of metastasized LNs, similar to Van Beek et al results,¹⁶ whereas CD19 expression was proportionally related to the number of LNs. This correlation was not significant in both and this was near to Ishigami S. et al study¹⁷ who explained that CD3 was not significantly related to lymph node involvement. CD19 expression did not correlate with grade of differentiation, near to Osada J. et al results.¹⁸ CD3 expression predominated with stage II & declined in

stage III carcinoma, whereas CD19 expression was more in stage III tumors. This was near to Ishigami S. et al. Osada J. et al. & Wang YX et al. results.^{17,18,19}

Conclusions:

In gastric carcinoma CD3 & CD19 expression was correlated with the LNs involvement & this explains the role of TILs in the spread of tumor. CD3 expressed well in the well differentiated & with the fewer LNs metastasizing tumors that explains the antitumor effect of cytotoxic T-lymphocytes as a defense mechanism. Statistically, there was no significant correlation between CD3 & CD19 markers expression with these studied clinicopathological parameters.

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