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ORIGINAL ARTICLE

Validity of Check Point Inhibitor (PD-1 and PD-L1) in Diagnosis of Gastric Adenocarcinoma Using Modified Tissue Elisa

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ABSTRACT

Background: Checkpoint inhibitors (PD-1, PD-L1) are revolutionizing the oncology disease management process. Despite the fact that gastric cancer is a common malignancy with a poor prognosis, relatively little attention has been drawn and attracted to the treatment and diagnosis of this disease by using checkpoint inhibitor. James P. Allison and Tasuku Honjo were awarded the the Nobel Prize in Physiology and Medicine for their developments in fundamental science enabling checkpoint inhibitor therapies. Checkpoint inhibitor overexpression has been related to gastric cancer progression and clues for developing therapeutic goals as well as may provide diagnostic markers for gastric cancer.

Aims: The aims of this study are firstly to detection of Checkpoint Inhibitors (PD-1, PD-L1) as novel diagnostics and prognostics markers first time in the world. Secondly, to investigate the validity of using (a modified tissue ELISA method) as a rapid, sensitive, low cost and specific diagnostic method in patients with gastric cancer.

Patients and methods:

Thirty gastric cancer patients were among patients who attending the Histopathology section -GIT hospital and Histopathology department- Teaching laboratories/ medical city teaching complex Baghdad / Ministry of Health, during the period from August 2020 to April 2021. Another 30 patients diagnosed with benign tumor, In addition, 30 apparently healthy people were chosen as a healthy control group. For these three groups, PD-1, PD-L1, using tissue ELISA technique was carried out.

Results:

The current study showed that the mean values of PD-1 in tissues of gastric cancer patients (133.413±53.126) was significantly higher (P=0.0001) in it in comparison to both benign tumor group (29.905±12.634) and control healthy group (21.775±12.489); for PD-L1 that the mean values of PD-L1 in tissues of gastric cancer patients (151.175±47.641) was significantly higher (P=0.0001) in it in comparison to both benign tumor group (72.565±9.945) and control healthy group (82.102±12.642); using receiver operating characteristic curve (ROC) area, which showed that area under the curve for PD-1 was (1.000) (p value 0.0001), while area under the curve for PDL-1 was (0.913) (p value 0.0001) and The cut off value for PD-1 associated with highest sensitivity and specificity (100%) was 40.2 ng/ml. The cut off value for PDL-1 associated with sensitivity (80%) and specificity (80%) was 51.6ng/ml.

Conclusions:

The present study showed that Checkpoint inhibitors (PD-1, PD-L1) values were significantly higher in patients affect with malignant gastric adenocarcinoma which may check a possible role of this marker in the progressing of the disease, furthermore the maximum sensitivity obtained from Checkpoint inhibitors (PD-1, PD-L1) was by using a cut off values equal to (40.2 ng/ml; 51.6 ng/ml) respectively. Therefore, Checkpoint inhibitors (PD-1, PD-L1) may be promising diagnostic tools especially at early stages and among patients at in promotion risk.

Keywords: Checkpoint inhibitors (PD-1, PD-L1), gastric cancer.

INTRODUCTION

Gastric cancers considered the most fatal malignancies worldwide and have a wide pathological and biological variety1. However, accurate molecular passageway of gastric carcinogenesis and clinical progression are yet to be elucidated. In order that a proteins appearance profile of gastric cancers is essential to provide a therapeutic targets on individual basis and diagnostic molecular markers for gastric cancer2.

Programmed cell death protein 1, also known as PD-1 and CD279 (differentiation cluster 279), is a cell surface protein that plays a role in controlling the response of the immune system to human body cells by down-regulating the immune system and promoting self-tolerance by suppressing the inflammatory activity of T cells. This prevents autoimmune disorders, but can also inhibit the destruction of cancer cells by the immune system 3. PD-1 is an immune checkpoint and two pathways guard against autoimmunity. First, in lymph nodes, it promotes apoptosis of antigen-specific T-cells. second, in regulatory T cells, apoptosis decreases 4, 5. Programmed death-ligand 1 (PD-L1) is a protein that is encoded in humans by the CD274 gene, also known as (CD274) differentiation cluster 274 or B7 homolog 1 (B7-H1) 6.

Binding and Signaling of each Checkpoint Inhibitors PD-1and PD-L1: To modulate activation or inhibition, PD-L1 binds to its receptor. Said et al. showed that PD-1, up-regulated on activated CD4 T-cells, can bind to monocyte-expressed PD-L1 and eventually induces the production of IL-10 7. The interaction of PD-L1 with its PD-1 receptor on T cells provides a signal that inhibits TCR-mediated IL-2 production and T cell proliferation. Furthermore, Checkpoint Inhibitors PD-1and PD-L1 released by the tumor might help the tumor to evade the immune surveillance 8.

MATERIALS AND METHODS

Subjects

Patients study group: Thirty gastric cancer patients with age range from (24-75) years with Mean±SD (57.3±12.4) were included in this study, another 30 patients with age range from (20-73) years with Mean±SD (53.3±14.6) diagnosed with benign tumor, these patients were diagnosed clinically, histopathologically and immunohistochemistry by specialists, they were among patients who attending the Histopathology department -GIT hospital and Histopathology department- Teaching laboratories/ medical city teaching complex Baghdad / Ministry of Health, during the period from August 2020 to April 2021. Ethical permission to conduct the research was obtained from this center. Thirty apparently healthy individuals, with age range from (8-76) years with Mean±SD (47.7±17.9) who have no clinical evidence of malignant diseases, were chosen. For these three groups, PD-1, PD-L1, using tissue ELISA technique was carried out.

Kits and reagents: Human Programmed Death 1 (PD-1) ELISA Kit and Human Programmed Death Ligand-1 (PD-L1/CD274) ELISA Kit These kits was a sandwich enzyme immunoassay.

Statistical analysis: Analysis of data was carried out using the available statistical package of SPSS-27 (Statistical Packages for Social Sciences- version 27) and Receiver Operating Characteristic "ROC" curve technique.

RESULTS

Assessment of check point inhibitor levels in comparison among these three groups: The distribution of PD-1 and PDL-1 in tissues of gastric cancer patients, benign tumor group and control healthy group are shown in (table 1) There was a statistically significant (P=0.0001) higher level of PD-1 in gastric malignancy group (133.413±53.126 ranging from 40.531 to 227.395) and benign gastric group (29.905±12.634 ranging from 0.744 to 63.053) in comparison to healthy control group (21.775±12.489 ranging from 1.419 to 39.950). The same finding for PDL-1 in comparison of the levels in the three groups; gastric cancer patients, benign tumor group and even with healthy control group (P=0.0001 & P=0.018 respectively) (table 1) (figure 1, 2).

Table 1: The ranges and mean values of PD-1 and PDL-1 in tissues of gastric cancer patients, benign tumor group and control healthy group.

	Malignant (n=30)	Benign (n=30)	Healthy control (n=30)	P value
PD-1	133.413±53.126 (40.531-227.395)	29.905±12.634 (0.744-43.053)	21.775±12.489 (1.419-39.950)	0.0001^
PDL-1	151.175±47.641 (49.278-232.667)	72.565±9.945 (60.001-94.285)	82.102±12.642 (54.090-100.823)	0.0001^
-Data were presented as Mean±SD (Range)				
#Significant difference between two independent means using Students-t-test at 0.05 level.				
^Significant difference among more than two independent means using ANOVA-test at 0.05 level.				

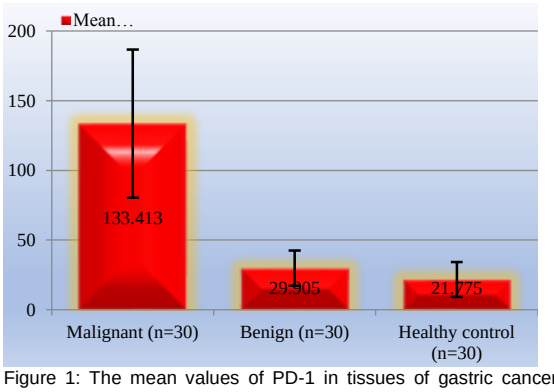

Figure 1: The mean values of PD-1 in tissues of gastric cancer patients, benign tumor group and control healthy group with p value (p <0.0001)

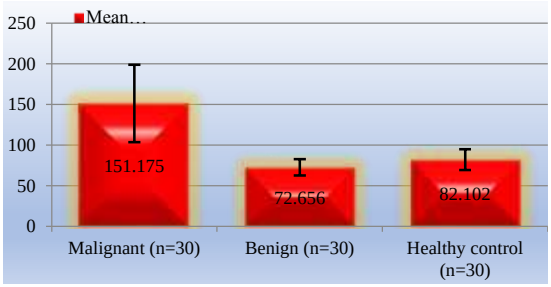

Figure 2: The mean values of PDL-1 in tissues of gastric cancer patients, benign tumor group and control healthy group with p value (p <0.0001)

Receiver operating characteristic curve (ROC) area and validity parameters of (PD-1, PDL-1and CTLA-4) in diagnosis of gastric cancer:

A- ROC area in differentiation among gastric cancer, benign tumor, and healthy control groups:

A.1 ROC area for gastric cancer patients and healthy control groups: Table 2. and figure 3. showed that area under the curve for PD-1 was (1.000) (p value 0.0001), while area under the curve for PDL-1 was (0.913) (p value 0.0001) and area under the curve for CTLA-4 was 0.631(p value 0.081). So that PD-1 value has the highest area under the curve (1.000) followed by PDL-1 value (0.913), and finally for CTLA-4 was (0.631).

B Validity parameters in differentiation among gastric cancer, benign tumor, and healthy control groups: The validity parameters of (PD-1, PDL-1and CTLA-4) in differentiating among gastric cancer, benign tumor, and healthy control groups were determined according to sensitivity, specificity.

B.1 Validity parameters for (PD-1, PDL-1and CTLA-4) (Table 3.9.): The cut off value for PD-1 associated with highest (perfect) sensitivity and specificity (100%) was 40.2 ng/ml. This cut-off value qualifies as the optimum (typical) cut-off value, being able to classify a tested individual into gastric cancer or healthy.

The cut off value for PDL-1 associated with sensitivity (80%) and specificity (80%) was 51. 6 ng/ml. This cut-off value qualifies as the optimum cut-off value, being able to classify a tested individual into gastric cancer or healthy.

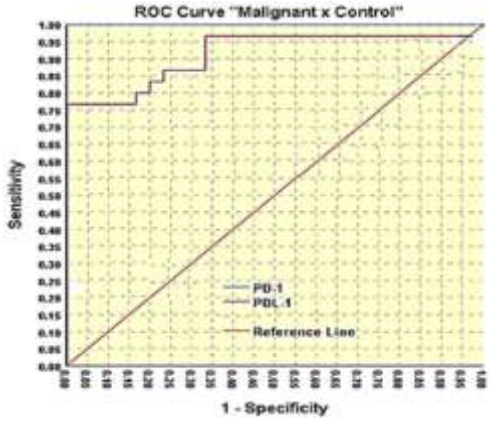

Figure 3: ROC curve showing the trade-off between sensitivity which is (rate of true positive) and 1-specificity which is (rate of false positive) for values of PD-1, PDL-1 between gastric cancer patients and control healthy group.

Table 2: ROC area for values of PD-1, PDL-1 for differentiating between gastric cancer patients and control healthy group.

Test Result Variables	Area Under the Curve (AUC)	Std. Error	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
PD-1	1.000	0.001	0.0001*	-	-
PDL-1	0.913	0.040	0.0001*	0.835	0.992
*Significant					

Table 3: Validity parameters for the studied values of PD-1, PDL-1 in differentiating between gastric cancer patients and control healthy group.

Test Result Variables	Positive if Greater Than or Equal To (cut-off value)	Sensitivity	Specificity
PD-1	31.84300	100	80.0
	39.80000	100	90.0
	40.24050	100	100
PDL-1	51.68400	96.7	0
	81.73050	96.7	50.0
	96.05550	80.0	80.0
	103.78250	76.7	100

DISCUSSION

Immunopathological finding:

A- Role of PD-1 and PDL-1 in gastric cancer: The current study showed that the mean values of PD-1 in tissues of gastric cancer patients (133.413±53.126) was significantly higher

(P=0.0001) in it in comparison to both benign tumor group (29.905±12.634) and control healthy group (21.775±12.489); for PD-L1 that the mean values of PD-L1 in tissues of gastric cancer patients (151.175±47.641) was significantly higher (P=0.0001) in it in comparison to both benign tumor group (72.565±9.945) and control healthy group (82.102±12.642).

These finding were in agreement with a study conducted by Zalba, S., and his colleagues (2020) who reported that the concentration of PD-1 and PD-L1 values range for the PD-1 was (2.5–125 ng/mL) and (0.11–3.125 ng/mL) for the PD-L1, were markedly increased in the tissues of patients with gastric cancer and in particular, those with matastasis 9.

Relative to benign and controls, the expression of PD-1 on tumor infiltrating T cells increased with disease progression. In vitro, T- cells induced PD-L1 expression on primary gastric adenocarcinoma epithelial cells in an IFN-γ-dependent manner, which in turn promoted T- cells apoptosis by PD-L1 up-regulation; so that the blocking of PD-L1 or IFN-γ-treated primary gastric adenocarcinoma epithelial cells could promote the apoptosis or necrosis of CD3+T cells reversed this effect 10. It was found that patients with cancer cells that overexpress PD-L1 had significantly poorer prognosis than those with cancer cells that under express PD-L1 10,11.

Here, the up-regulation of PD-L1 might be one negative feedback reaction, which is fully utilized by gastric adenocarcinoma cells to escape from immune surveillance; In addition, incubation with PD-L1 reduced primary gastric adenocarcinoma epithelial cell induced apoptosis of CD3+T cells and increased the cytotoxicity of CD3+T cells 10.

Validity parameters in differentiation among gastric cancer, benign tumor and healthy control groups:

Validity parameters for (PD-1 and PD-L1): This analysis were used to evaluate the performance of diagnostic tests and more generally for evaluating the accuracy of a statistical model that classifies subjects into one of two categories, diseased or not, with the area under the ROC curve (AUC) gives an idea about the usefulness of a tested parameter in differentiating between groups, the closer the area to one, the more useful it is in discrimination. So this ROC was used to determine the cut-off value for different parameters for diagnosis of gastric cancer as well as differentiating them from each benign and healthy control by the best test which is PD-1 as highly significant (P =0.0001), with Roc area 1.000 with high sensitivity and specificity. Also PD-L1 were highly significant (P= 0.0001) with ROC area 0.913 with high sensitivity and specificity (Figure 3).

In order to study the validity of check point inhibitor (PD-1, PDL-1) in differentiating between gastric cancer patients from healthy control group, the present study showed that in a patient with (ELISA)(PD-1, PDL-1) values above (40, 51.6) ng/ml respectively (cut off value) one can establish the diagnosis of gastric cancer with (95%) confident in clinical situation with Sensitivity and specificity of (PD-1, PDL-1,) by ELISA tests reached (100% , 100%),(96.7,0) respectively, and the area under the curve (AUC) for the (PD-1, PDL-1) tests was (1.0, 0.9,0.6) P-values(0.0001,0.0001) (Table 2).

These results were in agreement with Dai, L., et. al., 2021 how reported that The area under the ROC curve was calculated to evaluate the ability of PD-L1 in distinguishing the tumor sample from the normal sample and the AUC values of PD-L1 (0.543) in Gastrointestinal Cancer Patients 12.

Zheng, Z., et. al., 2014 revealed that the cut-off value of PD-L1 (0.59) ng/mL was best distinguished in patients without lymph node metastasis and with lymph node metastasis, which was used as a cut-off value for correlations and survival analysis, and the area under curve (AUC) value was 0.613 [95% confidence interval (95% CI), P=0.044] 13, So that the result of the present study is in agreement with previous findings with these documented that PD-L1 with its receptor PD-1, plays a critical role in suppressing T cell-based immunity and could mediate tumor immunosuppression. PD-1/PD-L1 interactions contribute to the maintenance of peripheral tolerance of self-antigens in normal hosts; in vitro study showed that PD-L1 specifically interacts with B7-1 to inhibit T and B cell activation and proliferation 13,14.

To further explore the existence of membranes PD-1, PDL-1 in gastric tissue and evaluate the pathological factor in human cancer, this study for the detection and quantification of PD-1, PDL-1 in advanced gastric cancer patients. Although the sPD-1, sPDL-1 levels in advanced gastric cancer which demonstrated in many studies is lower than that which reported in cancers in this present study, this indicates that the PD-L1 expression in tissue of advanced gastric cancer was much higher than that soluble in serum 13,15.

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