Involvement of Programmed Cell Death Receptor-1 in Pituitary Adenomas

Short title: Programmed Cell Death Receptor-1 in Pituitary Adenomas

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ABSTRACT

Background and Aim: Pituitary adenomas (PA) are the second most common intracranial neoplasms which can recur despite the treatment with traditional strategies. Cancer cells may evade from immune system cells pretending as host cells using the PD-1/PD-L1 axis which is used in the normal physiological function of T cells, and immunotherapy is based on the disruption of this axis via triggering T cell response. In this case-control study, we aimed to evaluate the PD-1 and PD-L1 protein levels in blood and tumor samples of patients with PA.

Methods: Study samples were obtained from 49 PA patients and 10 healthy controls for PD-1 and PD-L1 expression analysis. PDL1 expression in tissue and blood samples were evaluated by Real-Time Polymerase Chain Reaction. PD-1 levels were measured using a two-site sandwich ELISA kit in plasma samples.

Results: The mean level of expression for PDL1 protein was 60.902±100.171 pg/mL in PA tissues and 1±0 pg/mL in the control samples ($p<0.05$), whereas the mean expression level of PDL1 in circulation was 13.071±8.071 pg/mL in PA samples and 9.302±2.278 pg/mL in the controls ($p= 0.060$). The mean level of PD1 protein was 359.429±476.544 pg/mL in PA and 258.961±65.872 pg/mL in the control samples in the blood ($p= 0.135$).

Conclusion: PDL1 might serve as a promising candidate for cancer immunotherapy targeting the PD-1/PD-L1 pathway in patients with PA.

Keywords: Pituitary adenomas, Immunotherapy, Programmed cell death receptor 1, PD-1, PD-L1

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INTRODUCTION

The anterior pituitary gland has substantial roles both in physiological functions and human development and is responsible for the synthesis and secretion of a group of specific hormones. Pituitary adenomas (PA), are slow-growing benign tumors comprising approximately 20% of all brain tumors following meningiomas and gliomas, and the most frequently observed type of neuroendocrine tumors of the central nervous system (Asa et al., 2017; Daly et al., 2006; Cho et al., 2014)

In case of combating cancer, there are three advanced strategies (radiotherapy, chemotherapy, surgery) targeting directly the anatomic location of cancer cells to achieve success during the treatment. Owing to the increasing research in the past decades, immunotherapy has emerged as the fourth, and hence became a breakthrough in tumor management (Yang, 2015). Under normal physiological circumstances, the immune system is specialized to protect the host against infectious diseases and autoimmunity via recruiting several numbers of co-stimulatory and co-inhibitory receptors and their ligands, namely the immune checkpoints (ICPs) (Keir et al., 2008; Ribas 2012). The common ICPs proteins including cytotoxic T-lymphocyte-associated protein 4 and programmed cell death-1 (PD-1) maintain these processes through regulation of T-cell activities (Francisco et al., 2010).

Programmed cell death receptor 1 (PD-1), encoded by the PDCD1 gene, is a type I transmembrane protein receptor covering 5 exons and 288 amino acids, and has two ligands: PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2) (Keir et al., 2008; Ishida et al., 1992; Freeman et al., 2000; Dong et al., 1999; Tseng et al., 2001). PD-1 and PD-L1 axis takes a critical responsibility in physiological immune homeostasis, and mounting evidence suggests that tumor cells evade immunity using this axis to ultimately progress and metastasize (Keir et al., 2008; Keir et al., 2007; Sun et al., 2018).

In a cell-to-cell interaction through the PD-1/PD-L1 axis, T cell-mediated immune responses are negatively regulated limiting effector T-cell responses, and thus protecting cells from immune-mediated damage (Figure 1) (Keir et al., 2008). The inhibition of this interaction triggers immune responses in vitro and initiates antitumor activity (Fife et al., 2009). PD-L1, one of the two ligands of PD-1, is highly expressed in solid tumors and is associated with poor prognosis (Massi et al., 2014; Abiko et al., 2013; Wang et al., 2012). In the case of central nervous system tumors, PD-L1 was reported to be overexpressed in high-grade meningioma and glioblastoma, pointing out the possible checkpoint inhibition (Parsa et al., 2007; Du et al., 2015; Nduom et al., 2016).

Currently, there are three fully humanized IgG1 monoclonal antibodies, durvalumab, atezolizumab, and avelumab which specifically bind to PD-L1 to block PD-L1/PD-1 interaction, approved by the US Food and Drug Administration as PD-L1 inhibitors for cancer treatment (Figure 2) (Gong et al., 2018). However, when and how elevated PD-L1 expression is mediated during the cancer pathogenesis remains to be elucidated. Thus, in the present study, we aimed to compare the amount of PD-1 and PD-L1 between human pituitary adenoma tissues and the control groups due to the recent success of checkpoint inhibition immunotherapies in cancer treatment.

MATERIALS AND METHODS

This case-control study was conducted in accordance with the Helsinki statement, and the permission obtained from the Ethics Committee of the study hospital.

Of the eligible 68 patients for analysis, the study group consisted of 49 patients who have been operated with a preliminary diagnosis of pituitary adenoma in a single-center neurosurgery clinic between August 2018 and July 2020. Informed consent on the details of the study was obtained from all individuals.

Inclusion criteria included adult patients with pituitary lesions that require surgical intervention, pituitary lesion observed on an MRI scan, histopathologically proven disease,

a normal prolactin level for lesions <10 mm or a prolactin level < 100 ng/ml for lesions ≥ 10 mm in maximal tumor diameter.

Exclusion criteria include an unconfirmed postoperative histopathological diagnosis of pituitary adenoma, patients lost to follow up, patients with additional malignity, renal failure, hyperproliferative disease, and autoimmune disease.

The control group consisted of ten patients who underwent temporal lobectomy for the treatment of mesial temporal sclerosis (MTS) epilepsy, and plasma and brain tissue samples were obtained. All samples were kept in dry tubes in -80 ºC degrees until the day of analysis and transferred to the genetics laboratory.

Preoperative Evaluation

All patients were evaluated by the endocrinology clinic in the preoperative period. Plasma levels of growth hormone (GH), insulin-like growth factor-I (IGF-I), prolactin, adrenocorticotrophic hormone (ACTH), cortisol, thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), thyroxine (T4), luteinizing hormone (LH) and follicle-stimulating hormone (FSH), testosterone and estradiol were measured.

Dynamic contrast magnetic resonance imaging (MRI) was performed on all patients before the operation. Patients were classified into three subgroups depending on the MRI evaluation.

Knosp classification was based on the three lines drawn between the supraclinoid and intracavernous internal carotid artery (ICA). Grading was evaluated based on four degrees as follows: Grade 0, adenoma did not cross the medial intercarotid line; Grade 1: tumor extends to space between the medial tangent and the intercarotid line; Grade 2: tumor extends to space between the intercarotid line and the lateral tangent; Grade 3: tumor extends lateral to the lateral tangent whereas 3A defines superior and 3B defines inferior localization to the ICA; Grade 4: complete encasement of intracavernous ICA.

Maximum length of the tumor from the inferior to the superior at the sagittal view [Tumor size (TS)] was evaluated as Grade 1: length from the sellar base <10 mm; Grade 2: a tumor length of 10-20 mm; Grade 3: a tumor length of 20-30 mm; Grade 4: a tumor length of 30-40 mm; Grade 5: length > 40 mm.

Presence of the cavernous sinus invasion was assessed as the presence of a thin-line plan between the cavernous sinus medial wall and the lesion.

Pathological Evaluation

Histopathological and immunohistochemical examinations were performed by the same clinical pathologist at the study center. According to the evaluation, staining of pathological tissues was classified as GH, prolactin, ACTH, TSH, gonotrophic, polyhormonal and negative staining.

Genetic Analysis

PDL-1 isolation phase consisted of three steps as RNA isolation from tissue samples, synthesis of cDNA from RNA by reverse transcription, and demonstration of gene expression with Real-Time PCR.

PDL-1 RNA Isolation from Tissue Samples

PDL-1 RNA isolation from frozen tumor and control tissues was performed using Trizol Reagent. 1 ml Trizol ™ Reagent was added to 50-100 mg tissue sample, the samples were homogenized, and incubated for 5 minutes at room temperature. The sample was transferred to another 1.5 ml microcentrifuge tube and then centrifuged at 12-16,000xg for 10 minutes to remove insoluble particles. The transparent supernatant was transferred into a new Rnase-free 1.5 ml microcentrifuge tube. 200 µl of chloroform and 1 ml GENEzol™ Reagent was added on the tubes for resuspension.

The microcentrifuge tube was shaken vigorously for 10 seconds. To separate the phases, the sample was centrifuged at 12-16,000xg for 15 minutes at 4 °C. The aqueous supernatant was transferred to a new 1.5 ml microcentrifuge tube for the RNA precipitation step. The same volume of isopropanol was added to the aqueous phase. The tube was then inverted several times and mixed. The sample was incubated at room temperature for 10 minutes. The sample was centrifuged at 12-16,000xg for 10 minutes at 4 °C to form the RNA pellet. The supernatant was carefully

removed and discarded.
One ml of 70% ethanol was added and vortexed briefly to wash the RNA pellet. The sample was centrifuged at 12-16,000xg for 5 minutes at 4ºC, the supernatant was removed with a pipette. The RNA pellet was dried at room temperature for 5-10 minutes. 20-50 µl RNase-free water was added to resuspend the RNA pellet. It was incubated at 55-60 °C for 10-15 minutes. The isolated RNAs were stored in a -80 °C freezer.

RNA Quantification
For the determination of the quantity and purity of each isolated PDL-1 RNA, absorbance measures were performed at the wavelengths of 260 nm and 280 nm. 200 µl dH2O was used for blank, and the RNA amount was calculated with the formula of = A260 x 40 x 40 ng / µl, and the purity was calculated as the ratio of A260 / A280.

cDNA synthesis (RT-PCR)
One µg of total RNA, 1 µl of random primer and distilled water were mixed to obtain a total volume of 10 µl The mixture was incubated for 5 minutes at 65 ºC and then placed on ice. 5X Reaction buffer, 0.5 µl RNAase inhibitor, 2 µl dNTP mix, 2 µl reverse transcriptase were added with a final volume of 20 µl and placed into the PCR device for 60 minutes at 42 ºC and 5 minutes at 70 ºC.

Polymerase Chain Reaction
All synthesized cDNAs were tested using Human β-2 microglobulin gene primers. 1 µl forward and reverse primers, 2 µl MgCl, 0.2 µl Taq polymerase, 5 µlTaq polymerase buffer, and distilled water were added with a total volume of 50 µl. After the reaction was completed, a 2% agarose gel electrophoresis results were controlled under UV light.

Real-Time Polymerase Chain Reaction (RT-PCR)
After obtaining cDNA, PCR was performed in optimized conditions for PDL-1 and human β-2 microglobulin, using AppliedBiosystem 7500 Fast. 5 µl of Master mix, 1 µl of cDNA, 100 µl / µl of 0.5 µl forward and reverse primers, 3 µl of distilled H_2O was added with a final volume of 10 µl. PDL-1 and human β-2 microglobulin primers were used. Human β-2 microglobulin was used as a control. Primers were purchased from TaqMan (ThermoScientific). Each analysis was replicated twice.
RT-PCR results were evaluated according to the Livak method, each of the Ct values obtained for PDL-1 was subtracted from the average of that obtained for the human β-2 Microglobulin gene. Results were obtained in multiples of 1.

Blood plasma separation and PD-1 expression measurement
Venous blood samples were collected by venipuncture directly into plain and K_2EDTA-containing collection tubes. Plasma separation was performed after centrifugation of blood samples at 1500 g for 15 min. All specimens were kept frozen at –80 °C until the day of analysis. Samples were assayed for PD-L1 level measurement using a two-site sandwich ELISA kit (Invitrogen, Thermo Fisher Scientific, MA USA 02451) with the Triturus analyzer (Grifols International, Spain) according to the manufacturer's instructions.

Statistical analysis
SPSS 22.0 package program was used to evaluate the data. Results are presented as means ± standard deviation (SD), the minimum and maximum values, and frequency and percentage values. Mann-Whitney U test was used to compare two groups that are not normally distributed. Kruskal-Wallis test was used to compare more than two groups. Spearman's correlation test was used to determine the relationship between two continuous variables. A p value <0.05 was accepted as statistically significant.

RESULTS

Demographic data of the study subjects are presented in Table 1. The study group consisted 49 patients (22 F, 27 M) and 10 controls (5 F, 5 M). Five patients had Grade 0 PA, whereas 10 had Grade 1, nine had Grade 2, twlve had Grade 3, and 13 had Grade 4 diasease according to the Knosp classification. Preoperative hormone profile demostrated presence of non-secreting adenomas in 24 patients (49%). Hormone staining turned negative for nine patients, and 40 patients were positive for pituitary hormones. The final status of the patients was evaluated as remission in 29 cases (56.5%, whereas residual tumor was detected in.

The mean level of PDL1 protein was 13.071±8.071 pg/mL in PA samples and 9.302±2.278 pg/mL in the controls for the blood samples (p= 0.060).

The mean level of PD1 protein was 359.429±476.544 pg/mL in PA samples and 258.961±65.872 pg/mL in the controls for the blood samples (p= 0.135).

According to the RT-PCR analysis, the mean level of PD-L1 protein in PA tissues was 60.902±100.171 pg/mL whereas the mean PD-L1 protein level 1±0 pg/mL in the control samples ($p<0.05$) (Table 2).

The Spearman's correlation test yielded a nonsignificant correlation between the study parameters, PDL1 expression in tissue samples, PDL1 and PD-1 expression in blood samples ($p> 0.05$) (Table 3).

While we investigated the relationship between the demographic characteristics of the patients and PDL1 expression in blood samples of the PA group, the mean level of PDL1 expession was significantly higher in the male subjects (p= 0.02). The difference was not statistically significant for other variables including the aggressiveness and final status of the tumor, hormone staining, and preoperative hormone profile (Table 4).

The comparison of gender, aggressiveness and final status of the tumor, hormone staining, and preoperative hormone profile for the tissue expression levels of PD-L1 protein and blood expression levels of the PD-1 protein did not yield statistically significant results between the patient and control samples (Tables 5 and 6).

DISCUSSION

Fundamental findings over the last two decades have initiated to brighten the complex interaction between cancer cells and the immune system. In their groundbreaking survey of cancer immunotherapy, Dunn et al. claimed that the evolution of the tumor cells to evade immune responses is a necessarily sustained process to keep growing (Dunn et al., 2004). The rapid development of immunotherapeutic monoclonal antibodies for PD-L1 and PD-1 proteins led to a new and promising strategy to battle particularly with the solid tumors such as pituitary adenomas.

Despite the presence of promising laboratory results and several positive clinical data worldwide, the overall clinical benefits seem to be reduced because of acquired and/or intrinsic resistance to the therapy (Sharma et al., 2017). Besides, the relatively larger size of the monoclonal antibodies given may prevent these proteins from penetration into the tumor microenvironment, thus minimizing the efficiency of the therapy (Lee et al., 2010).

In the present study, we investigated the expression patterns of PD-L1 and PD-1 proteins in the blood and tissue samples of patients with pituitary adenomas and compared their levels of expression with the control subjects in order to evaluate whether PD-L1/PD-1 axis-dependent immunotherapy is applicable for PA patients. We found that the PD-L1 protein expression in PA patient samples was upregulated, unlike the control samples. We could not find any statistically significant differences between the blood samples of the patients and the controls.

Our findings are in consistent with the previous studies with pituitary neuroendocrine tumors and adenomas (Mei et al., 2016; Suteau et al., 2020). In their cases of PA, Mei (2016) revealed elevated PD-L1 expression in human functioning adenomas but not in nonfunctioning adenomas indicating the presence of an immune response against the PA. They further concluded that, the mRNA and protein expressions of PD-L1 in pituitary tumors were independent from the tumor hormone secretion, proliferative index, aggressiveness level, or recurrence status, supporting our findings in the current study.

Although the preliminary results were encouraging, the PD-L1/PD-1 axis-dependent immunotherapies in hematological malignancies were not successful due to the other interfering genomic abnormalities. Increased PD-L1 expression was observable in classical Hodgkin lymphoma cases, whereas the results were heterogeneous for non-

Hodgkin lymphomas (Green et al., 2010; Zinzani et al., 2017; Kiyasu et al., 2015). Moreover, the mutation diversity, for instance JAK2 inactivation mutations lead to acquired resistance in numerous tumors, resulting in unfavored outcomes including the suppression of PD-L1 expression (Shin et al., 2017). Also, PD-1/PD-L1 is also located on normal cells to maintain host immunity, thus the inhibition of normal PD-1/PD-L1 functions may lead to the depletion of the cells that are intended to be functioning (Horita et al., 2017; Tan et al., 2016). Finally, the activation of the pro-survival signal pathways such as PI3K/Akt and MAPK and the transcriptional factors such as NF-κB and HIF-1 trigger cell proliferation and reduce apoptosis through PD-L1 up-regulation, thus mediating not only cancer development but also immune modulation, and anti-tumor immunity (Chen et al., 2016; Zhao et al., 2017).

Our study has several limitations to declare. Firstly, we only measured the expression levels in terms of protein both for the tissue and blood samples, and implementation of an mRNA-based study might give different results. Furthermore, we did not compare the study variables with the microscopic tissue-level immune response against the tumor cells, and evaluation of the level of lymphocyte infiltration surrounding the tumor tissue might provide a more comprehensive understanding on a possible upregulation of PD-1/PD-L1 in PA cells.

In conclusion, since the current approaches for targeting PD-L1 may prohibit its physiological functions in T cells, the understanding of the regulatory mechanism of PD-L1 expression should be deepened to identify which patients are likely to have a response, to determine which type of tumor might be cured with this PD-L1/PD-1 axis, and to find the appropriate clinical dose. Because of the difficulty of the early diagnosis of the PAs due to their general rarity and symptomless nature, the traditional strategies may be too late to cure the disease, thus the immunotherapeutic agents with fast response ability may open a new avenue for PA treatment. Taking into account these considerations, our findings suggest PD-L1 based immunotherapy as a promising treatment candidate for pituitary adenomas. However, further clinical studies on the PD-L1/PD-1 axis are called for in larger study samples.

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The study was approved by the institutional ethical board of the Bakirkoy Research and Training, Hospital for Psychiatry, Neurology, and Neurosurgery. Informed consent was obtained from study participants.

Author contributions:

OG: Conception or design of the work. Data collection. Data analysis and interpretation. Critical revision of the article. Final approval of the version to be published.

BE: Conception or design of the work. Data collection. Data analysis and interpretation. Drafting the article.

BC: Data collection. Data analysis and interpretation. Drafting the article.

SCD: Conception or design of the work. Data analysis and interpretation. Critical revision of the article. Final approval of the version to be published.

ME: Data collection.

ASA: Conception or design of the work. Critical revision of the article. Final approval of the version to be published.

MK: Data collection. Drafting the article.

LSP: Data collection. Drafting the article.

OT: Conception or design of the work. Data analysis and interpretation. Critical revision of the article. Final approval of the version to be published.

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Table 1. The demographic characteristics of the samples

N=69	**n**	**%**
Group		
Patient Samples	49	71
Control (Blood)	10	43965
Control (Tissue)	10	43965
Gender		
Female	22	44.9
Male	27	55.1
Control Tissue Gender		
Female	5	50
Male	5	50
Knosp Classification		
0	5	43871
1	10	43941

2	9	43939
3	12	43975
4	13	43977
Hardy's radiological classification		
0	1	2
1	5	43871
2	12	43975
3	11	43943
4	7	43904
5	13	43977
Aggressiveness		
Invasive	27	55.1
Non-invasive	22	44.9
Final Status		
Remission	26	56.5
Residue	20	43.5
Hormone staining		
Negative	9	43939
ACTH (pg/mL)	6	43873
GH (ng/mL)	9	43939
Gonadotropin (mIU/mL)	14	44010
Plurihor monal cells	6	43873
Prolaktin (ng/mL)	5	43871
TSH (mU/L)	-	-
Preoperative hormone profile		
Non-functional	24	49
ACTH (pg/mL)	5	43871
GH (ng/mL)	14	44010
Gonadotropin (mIU/mL)	5	43871
Prolaktin (ng/mL)	-	-
TSH (mU/L)	1	2

Table 2. The comparison of pituitary PD-L1 and PD-1 values (Blood) and PD-L1 values (Tissue) between the patient and control groups

N=59		Mean±SD	Min-Max	Z
				p-value
PDL1 Blood	**N**			
Patient	49	13.071±8.728	5.26-49.79	-1.879

Control	10	9.302±2.278	6.08-12.12	0.060
Total	59	12.432±8.116	5.26-49.79	
PD1 Blood				
Patient	49	359.429±476.544	99.44-2075.57	-1.495
Control	10	258.961±65.872	197.68-418.38	0.135
Total	59	342.401±435.957	99.44-2075.57	
N=59		**PDL1 TISSUE**		
		Mean±SD	**Min-Max**	**t**
				p-value
PDL1 Tissue	**N**			
Patient	49	60.902±100.171	1.27-528.95	-4.962
Control	10	1±0	1-1	**0.000**
Total	59	44.562±71.309	0.10-320.07	

Table 3. The evaluation of the correlation between PD-L1 tissue, PDL1-blood, and PD-1 blood Levels

		PDL1 BLOOD	PD1 BLOOD	PDL1 TISSUE
PDL1 Blood	**r**	1	35	-0.001
	p		0.793	0.995
	N	59	59	49
PD1 Blood	**r**	0.035	1	0.177
	p	0.793		0.223
	N	59	59	49
PDL1 Tissue	**r**	-0.001	0.177	1
	p	0.995	0.223	
	N	49	49	59

Table 4. The comparison of pituitary PD-L1 (Blood) values with the patient's demographic and biochemical features

N=69	**PDL1-Blood**		**z/X²**
	Mean±SD	**Min-Max**	**p-value**
Gender			
Female	9.666±2.612	5.26-14.16	-2.253
Male	15.846±10.831	6.81-49.79	**0.024**
Aggressiveness			
Invasive	12.858±7.019	5.41-40.02	-0.864
Non-invasive	13.332±10.63	5.26-49.79	0.387
Final Status			
Remission	12.282±8.188	5.26-49.79	-0.465
Residue	14.679±9.871	6.61-40.02	0.642
Hormone staining			
Negative	11.214±10.703	5.41-39.35	0.9284
ACTH (pg/mL)	16.227±16.477	7.91-49.79	

GH (ng/mL)	10.319±3.136	5.26-14.91	
Gonadotropin (mIU/mL)	15.465±8.846	8.48-40.02	
Plurihormonal cells	12.365±2.234	9.05-14.80	
Prolactin (ng/mL)	11.724±1.585	9.22-13.08	0.098
TSH (mU/L)	-	-	
Preoperative hormone profile			
Non-functional	13.642±9.434	5.41-40.02	
ACTH (pg/mL)	17.479±18.101	7.91-49.79	2.905
GH (ng/mL)	10.876±2.833	5.26-14.91	
Gonadotropin (mIU/mL)	-	-	
Prolactin (ng/mL)	12.841±1.322	11.14-14.80	0.584
TSH (mU/L)	9.215±-	9.22-9.22	

Table 5. The comparison of pituitary PD-L1 tissue values with the patient's demographic and biochemical features

N=69	PDL1-Tissue		z/X^2
	Mean±SD	Min-Max	p-value
Gender			
Female	77.674±128.371	1.27-528.95	-.283
Male	47.236±69.158	1.81-340.68	0.777
Aggressiveness			
Invasive	55.473±87.569	1.27-340.68	-0.663
Non-invasive	67.564±115.572	2.29-528.95	0.577
Final Status			
Remission	79.79±121.562	1.81-528.95	-1.152
Residue	41.815±69.217	1.27-322.84	0.249
Hormone staining			
Negative	61.906±99.731	4.36-322.84	
ACTH (pg/mL)	21.065±17.109	1.81-40.70	
GH (ng/mL)	68.823±81.926	8.92-229.92	1.201
Gonadotropin (mIU/mL)	57.676±89.444	1.27-340.68	
Plurihormonal cells	112.32±206.435	3.40-528.95	
Prolactin (ng/mL)	39.972±19.63	10.54-62.77	0.753
TSH (mU/L)	-	-	
Preoperative hormone profile			
Non-functional	56.935±90.131	1.27-340.68	
ACTH (pg/mL)	24.916±15.958	2.29-40.70	2.317
GH (ng/mL)	86.962±145.038	3.4-528.95	
Gonadotropin (mIU/mL)	-	-	0.804
Prolactin (ng/mL)	45.368±25.514	10.54-75.85	

TSH (mU/L)	48.87±-	48.87-48.87	

Table 6. The comparison of pituitary PD-1 blood values with the patient's demographic and biochemical features

N=69	PD1-Blood		z/X²
	Mean±SD	Min-Max	p-value
Gender			
Female	346.975±540.717	99.44-2047.78	-1.879
Male	369.578±427.625	136.11-2075.57	0.060
Aggressiveness			
Invasive	303.139±367.163	136.11-2075.57	-1.307
Non-invasive	428.513±585.741	99.44-2047.78	0.191
Final Status			
Remission	375.691±522.487	99.44-2047.78	-.820
Residue	359.811±458.967	108.48-2075.57	0.412
Hormone staining			
Negative	312.058±309.448	136.11-1125.34	8.222
ACTH (pg/mL)	682.444±762.739	181.77-2047.78	
GH (ng/mL)	403.558±604.971	108.48-1975.10	
Gonadotropin (mIU/mL)	357.309±502.961	138.92-2075.57	
Plurihor monal cells	166.837±53.705	99.44-257.09	0.116
Prolactin (ng/mL)	214.698±29.758	195.84-267.53	
TSH (mU/L)	-	-	
Preoperative hormone profile			
Non-functional	335.354±420.965	136.11-2075.57	7.073
ACTH (pg/mL)	771.406±817.226	181.77-2047.78	
GH (ng/mL)	319.928±489.771	99.44-1975.10	
Gonadotropin (mIU/mL)	-	-	0.132
Prolactin (ng/mL)	204.63±40.641	154.05-267.53	
TSH (mU/L)	204.393±-	204.39-204.39	

Figure Legends

Figure 1. The PD-1 / PD-L1 axis. A) T cells with their PD-1 receptor can interact with the host cells' PD-L1 ligand to maintain immunity. B) Cancer cells can evade from the immunity response owing to their PD-L1 ligand on their cell membrane which makes them being recognized as host cells by T cells.

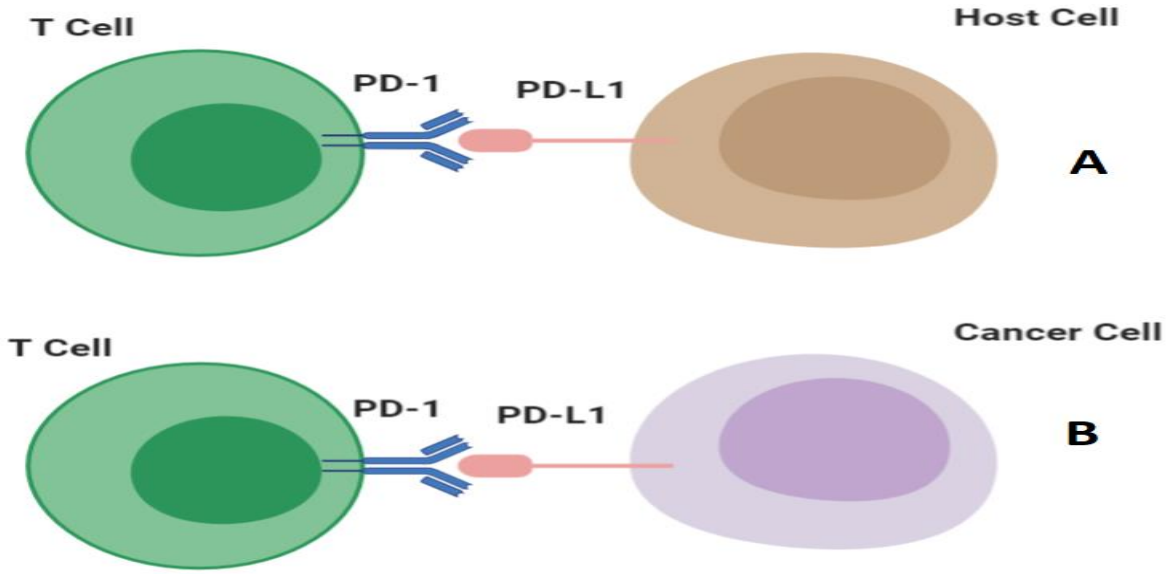

Figure 2. Immunotherapy can be an option to treat cancer with anti-PD-1 and anti-PD-L1 agents which could break the PD-1 / PD-L1 axis leading to a fast immune response.