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Correlation Between AP-1 and PDL-1 Gene Countenance in Patients with Hodgkin's Lymphoma

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Abstract

EBV infection induces the countenance of PD-L1, which limits antitumor T cell responses by different and potential mechanisms, Here we highlight the estimation level of the AP1 transcription factor that acts as a double-edged sword with both oncogenic and tumor suppressive activities by control on the level of PD-L1 checkpoints in EBV-infected Hodgkin lymphoma patients. To our knowledge, This is the first Iraqi research attempt to explore the detailed mechanism of PDL1 up-regulation by AP1 which is induced by EBV infection. The research including of 23 cases Hodgkin lymphoma and 10 healthy control, after diagnosis of EBV VCA IgG by using ELISA assay. Our result indicated that, the level EBV VCA IgG was positive in (10) cases Hodgkin lymphoma at a percentage (43.47%) while the absence of this antibody in control group at a rate (0.0%). Also, the mean of PDL-1gene significantly higher in Hodgkin lymphoma compared with mean of PD-L1 (2.2±1.84) in control , as well as , the statistical significant increased value of AP-1 in Hodgkin lymphoma (1.37 ±0.94) compared with control (1.07±0.38) , addition in this research we found significant correlation between AP-1and PDL-1 in EBV + Hodgkin lymphoma patients with (r= 0.40) at significant ($p \leq 0.05$) .

Key Words: EBV, AP-1, PDL-1, Hodgkin.

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INTRODUCTION

Infection with this virus leads to the stimulation of resting B lymphocytes to multiply to maintain cellular replication. Meanwhile, EBV expresses lytic genes and latent genes during the infection cycle. In the period of EBV lytic reactivation, different types of genes are expressed one of them is known as viral capsid antigen (VCA) which classified as early gene. also, the pattern of EBV latent protein countenance differs in these tumors like EBNA1 and (LMP1 and LMP2A/B) thst are expressed in Hodgkin's lymphoma (1 , 2).However as immune response to this virus already the immune system try to deal with that by using a variety of means to eradicate or limit the spread of virus , on the other hand the virus works to circumvent the immune system. this evading mechanism is an emerging hallmark for tumor survival and development. Programmed cell death receptor1 (also called PD-1 or CD279) by it reducing T cell function that causes autoimmunity (4)

Hodgkin lymphoma cells is the malignancy of B-cell that are surrounded with a various variety immune checkpoint ligands as well as transmembrane receptors mediating inhibition cytotoxic T cells and type 1 helper T-cell subsets. like Cytotoxic T-lymphocyte antigen 4 (CTLA-4), membrane-bound TGF- β, also PD-L1 (B7-H1) countenance are some of the major T-reg contact mediators acting in this process of exhaustion .PD-L1 immunosuppressive is also expressing by many macrophages in the tumor microenvironment , thus enhanced PD-1/PD-L1 signaling in Hodgkin lymphoma allow malignant cells in these lymphomas evading immune-surveillance and interact with immune cells in the microenvironment of cancer for survival and growth in HL (5,6) . in the studies of (7) show that the hallmark cells of Hodgkin lymphoma, commonly over express PD-1, where LMP1 gene induce elevation of PD-L1 countenance by AP-1-associated transcriptional activities(9).

MATERIALS AND METHODS

Study groups

The five ml of blood was collected intravenously and taken from 23 patients

with Hodgkin lymphoma, 13 female and 10 male the age range (18-65) , that is collection from in Middle Euphrates cancer center in Najaf AL-Ashraf during the period from (December 2021 - November 2022). control group consisted of (10 blood sample) (5 males and 5 females) with age range (20-60 year), the study group undergo to the ELISA test to Diagnosis of EBV depending on detecting EBV-,IgG VCA in the serum patients by utilizing ELISA kits provided via company Demeditec/Germany.

Overall RNA insertion

Overall RNA insertion from blood samples was extracted by utilizing total RNA extraction kit /company Acusol (TM)/Bioneer, Korea .according to the procedure provided by the manufacturer. Then all extracted RNA sample was preserved at -80 C° .

Primer of target gene

The briefings of objective gene AP1, PDL1 and GAPDH were formerly premeditated by used NCBI-Gene Bank catalogue also Briefing 3 design online, buttressed by company Macrogen, Korea, as following :

Amplicon	Sequence		Primer
6406.1g/mole	5-GGTGGGATAAGACCCCCTCA-3	F	**AP1**
-15	3-TCCTGCCTGCATAGCAATAGG-5	R	**Gene**
6099.9g/mole	5-GTGGCATCCAAGATACAAACTCAA-3	F	**PDL1 gene**
-16	5-TCCTTCCTCTTGTCACGCTCA-3	R	
136bp (17)	5-ATGGGGAAGGTGAAGGTCG-3	F	**GAPDH**
	5-GGGTCATTGATGGCAACAATATC-3	R	**Gene**

Real-time RT-qPCR technology

Real-time (RT-qPCR) was premeditated to ration qualified quantification changes in gene countenance levels to AP1, PDL1, based on qPCR principal mix had prepare based on NEXpro™ qPCR Master Mix kit abounding by the company and vessel on SYBER lime colorant , this technique was performed dependent on the method described by Wang and Hardy (18)the procedure thermocycler conditions were applied as follow(table2).

Tab.2. Thermocycler steps for cDNA synthesis.

Reprise cycle	Period	Hotness	qPCR step
1	5min	95 °C	**Preliminary Denaturation**
45	20 sec	95 °C	**Denaturation**
	30 sec	60 °C	**Strengthening\ Leeway**
			Revealing(scan)

Data analysis of qRT-PCR

The fallouts data of q RT-PCR for objective and GAPDH genes were scrutinized by using reference method that described by (19),Which calculates the relative of the fold changes in cDNA target gene and normalized with The GAPDH genes by using the 2-ΔΔCt
method .

Statistical analysis

The analyzed statistically was done through using SPSS (statistical package for social sciences) VR. 23 by using T test ,the mean and Sd was used to compared between group study and control at significant (≤ 0.05) , also person correlation use to described the relation between target gene at significant (≤ 0.05) (20).

Results and Discussion

Serological diagnosis of EBV

The serological diagnosis results of EBV (VCA) IgG done by used ELISA assay of the study group and control . The levels of EBV (VCA) IgG was positive in (10 of 23) cases in Hodgkin lymphoma at a percentage (43.47%) while the absence of this antibody in control groups at a rate (0.0%). Several studies in Iraq indicated the role of EBV positive in

Hodgkin lymphoma like studies of (27) show that EBV LMP-1 was positive in about 15 out of 40 cases at percentage of 37.5% in patients with Hodgkin's lymphoma. while , EBV was positive in 25 from 40 cases of Hodgkin's lymphoma at percentage (62.5%) (28) .

Measurement of Quantification AP1 and PDL1 gene manifestation of RT-qPCR

Assessable gene countenance of (RT-qPCR) was premeditated based on the proportion of the statistics of verge series (CT) conspiracy strengthening to the target genes besides GAPDH housekeeping gene. the results of qPCR strengthening subversion of GAPDH gene had shown no difference, where observed in the control CT range (23-24) also, the patient groups show CT range (23- 24) as (Figure 1) as well as, The upshots of q PCR amplification plot for target genes PDL1 and AP1 revealed alteration in the CT ratio when associated between persevering groups and rheostat as in (Figure 2 ,Figure 3).

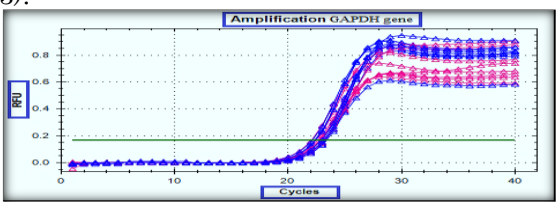

Fig.1. Diagram plot amplification q PCR of GAPDH gene. Where, red Hodgkin lymphoma and blue plot referred to control group.

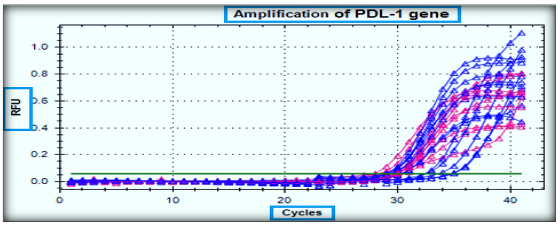

Fig.2. Diagram plot amplification q PCR of PD-L1 gene. Where, red Hodgkin lymphoma and blue plot blue plot referred Control group.

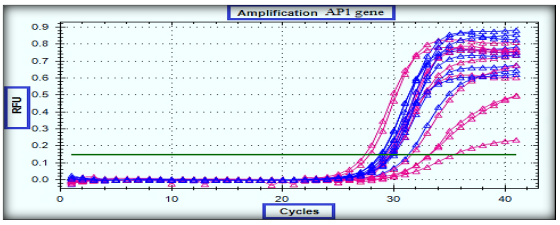

Fig.3. Diagram plot amplification q PCR of AP1 gene. Where, red Hodgkin lymphoma and blue plot blue plot referred Control group.

Estimation the value of PD-L1gene countenance in the training assembly

Our fallouts specified in (Figure4) There was significant statically modifications between developed nasty PDL-1gene value (4.16±2.26) in Hodgkin lymphoma and mean PD-L1 value (2.2±1.84) in control as (Figure 4) .

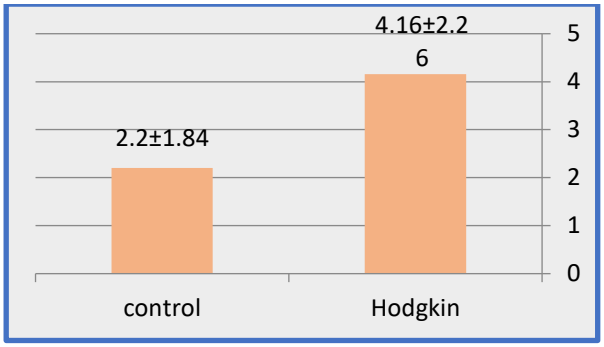

Fig.4. Gene countenance of PDL1 in Study group.

Numerous studies explained that EBV infection of malignant lymphocytes by uses many mechanisms to circumvent immune eradication to establish latency include increased production of latent genes like LMP1 that activation over-countenance of PD-L1in activity in B cells (35 , 36) addition, EBNA1 have role important in latency of EBV infection such studies of (37) explained that EBNA1 could induce PD-L1 activity in lymphoma cells . therefore, EBV uses PD-L1 to evade immune state through a decrease in MHC class I countenance, it is presumed immune axis of checkpoint PD-1/PD-L1/2 (29, 8). I think that PD-L1 over countenance in EBV + HL important to allow the virus evading from immunity system and increased cancer cell growth and progression disease. The tumor microenvironment promotes not only malignant cells but as well as cytokines and antigen-presenting cells (APCs) like dendritic cells, and macrophages. These APCs prevent activation of CD8+ T cells function by PD-L1 expressing and through establishing interference with PD-1 on the surface of CD8 T cells, and at the same time, they can release a substantial number of cytokines like IFN-γ, IL-10, and IL-2 that indirectly stimulate PD-L1 countenance in tumor cells and APCs(38, 39) . thus Even in EBV negative cHL , the countenance of PD-L1 on HL surface is still high. these allow the interaction of PD-1-PD-L1 triggers the prevention of Cytotoxicity T- cell function. also TAM (tumor-associated macrophages) express a high surface PD-L1, thus inducing PD-1-PD-L1 axis immune escape. (40) ,thus the hallmark cells of Hodgkin lymphoma, commonly over express PD-1 ligands, (7) ,

There are several studies revealed that Hodgkin lymphoma show a co-countenance of PD-L1, therefore in the tumor microenvironment the PD-L1 is richly countenance on infiltrating lymphocytes like macrophage lineage, and monocytes. And this status is very important in HL . (41, 42). addition NK cells in HL express elevated levels of PD1, and reverse signaling by PD-L1 in HL that supports the survival and proliferation of these cell (43) also, in the studies of (44). revealed high countenance of PDL1 in cHL samples at percentage (80% in cHL and 75% in nodular lymphocyte-predominant HL (NLPHL).

The study by (35) identified that the (PDL1/B7-HI/CD274) is a contributor to the immunosuppressive microenvironment of cHL, also reveald assessing a potential role for EBV in cHL PD-L1 countenance thus PD-L1 countenance in primary cHLs and viral infection can induce PD-L1. also, the recent studies of (45) indicated That high PD-L1 countenance is predicted to stimulate inhibition of the immune upon interaction with receptors PD-1 on effector T cells, also HL cells increase the countenance of PD-L1 levels, and as a result, the infection of EBV leads to promoting proliferation and survival of cancer cells also enable immune evasion of these cells. while the studies of (46) show that the countenances of PD-1 and PD-L1 were high in 17 cases with HL by immune-histochemistry. While the studies of (47) revealed PD-1 receptor countenance on EBV-specific CD8 T cells .

Estimation the value of AP-1gene countenance in the study group.

Our result indicated that the mean of AP-1 significantly higher in Hodgkin lymphoma (1.37 ±0.94) compared with control (1.07±0.38) . AP-1 is a dimeric complex that has a crucial mediator in propagating and maintaining the phenotype of the tumor, these interfering are significant for inducing tumor-stroma interaction, adhesion, and metastasis. Thus, at the cellular level, AP-1 regulates cell proliferation by activating or depressing key components' countenance to the machinery of the cell cycle. (48) , moreover when a deregulated, either through up regulation or downregulation, AP-1 induces tumorigenesis basis on the cellular context. (49), therefore AP-1 is an implication in main cancer-related pathways, like inflammation, differentiation, the migration of cellular, metastasis, angiogenesis, and the healing of wounds (50) , also , AP-1 is deregulated in solid tumors and hematological malignancies. where, it plays a role important in lymphoid malignancies, such as Non-Hodgkin Lymphoma (NHL), sub-type peripheral T-cell lymphoma (PTCL), and Classical Hodgkin Lymphoma (CHL) (51) . Many studies had highlighted the role of AP1 protein in lymphoid malignancies like the studies of [52 ,53,54]. indicated AP1 proteins play in the pathobiology of lymphomas thus the elevated countenance of these transcription factors in cHL induces proliferation, suppressing apoptosis, and escaping the host immune response (55). while other cancer can be associated with the countenance of AP-1 proteins like the lung (56), gastrointestinal tract (57) , breast (58), brain (59) , ovaries (60) , skin (61) , and bone (62). However, AP-1 has been revealed for regulating many ranges of cellular processes, such as proliferation, differentiation, and apoptosis (50).

Many mechanisms that EBV is used to induce of AP1 in the tumor cell, where the reactivation of the EBV from latency into the lytic cycle is orchestrated by ZEBRA (BZLF1), which is a homolog of the AP-1 proteins and mimics some functions of the AP1 protein that are instrumental in the activation of quiescent EBV-infected B cells, thus AP-1 acts as a master regulator of the switch needed to induce EBV lytic DNA replication in latently infected B cells. through activation of BZLF1 , these viral proteins have a role crucial in both establishing latency and to evade from it, also, it stimulates the ability of the infected resting B cells to proliferate (63 , 64). Another mechanism that the virus utilizes for activation of AP-1 is the production of latent proteins encoded by EBV like LMP1 and EBNA1, LMP1 is a protein that acts as an oncogene and causes immortalization and transformation of the latently infected cells. (65) , also, this protein increases AP-1 activity and changes critical protein countenance involved in the anti-apoptosis, proliferation, and invasion of cells and eventually leading to tumorigenesis (66). where several studies referred to AP-1 activation that mediates many downstream oncogenic effects of EBV LMP1 (67, 68,69) . addition the increasing activate of AP-1 is afforded by EBNA1, that have a role in promoting the countenance and activation of

AP-1 which have potent oncogenic potential (70), we think, increased the activity of AP-1 by EBV latent protein may induces modulation of immunity and allows virus replication in the infected B cell, which causes developed of the disease.

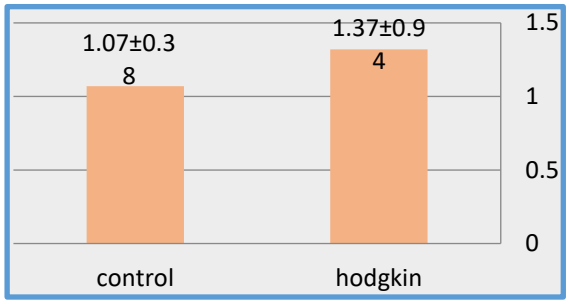

Fig.4. Gene countenance of AP1 in Study group.

Correlation between Ap1 and PDL1 in patients

The consequence in this research showed that there was a substantial statically association between gene countenance of AP-1 and PDL-1 in Hodgkin's lymphoma with ($r = 0.40$) at significant ($p \leq 0.05$). The immune escape of the cancer is an emerging hallmark of tumors . also, the many cellular mechanisms for inducing the countenance of immune evasion molecules in tumors , (71,72). Althought the countenance of PD-L1 in other malignancies with constitutive AP-1 activity such as anaplastic large cell lymphoma (73, 74, 75) and melanoma (76) in additional EBV-associated tumors (77, 78). but in hodgkin lymphoma. the immunosuppressive microenvironment encirclement the malignant Reed/Sternberg RS cells revealing numerous immune evasion mechanisms (71) where inducing PD-L1 activation of AP-1 via HRS cells results in increased countenance of PDL1 and/or PD-L2, besides interfering of these linking with PD-1 effector T cells depressed an effective anti-tumor immune response. (79), thus in this lymphoma PD-L1 genes correlated with immune evade, while AP-1 is essential to shape the cHL tumor microenvironment, also, the AP-1 components like (c-Jun, JunB) regulated genes that are important in the pathobiology of cHL and have essential roles in inducing proliferation in this lymphoma. where both c-Jun and also, JunB promotes co-inhibitory immune checkpoints PD-L1 (80) . Several studies revealed that , the AP1 component bind the PDL1 promoter during EBV-infected Hodgkin's lymphoma. (81 , 82), where The countenance level of PD-L1 levels increased as a result diversity of EBV protein products such as LMP1 which is an important oncogene that induces up regulates PD-L1 countenance through AP1 (83) , also LMP1 mimics CD40 signaling and therefore amplifies the over countenance of PD-L1/L2, by AP-1. This observation avoidance of viral clearance also immune evade of Hodgkin and Reed/Sternberg (HRS) cells (84) addition EBNA1 enhance PD-L1 promoter activity (37), we think our data revealed that during EBV latent infected , the activity of AP1 gene is important to control on immune response by bind to PDL1 , this phenomena allow the virus replicated and tumor growth.

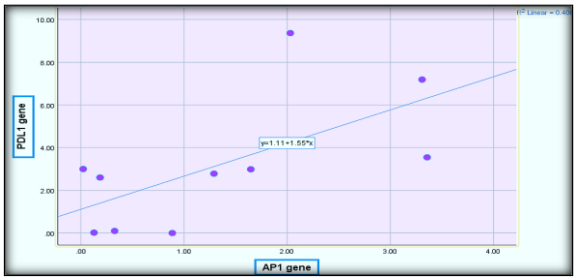

Fig.5. correlation between gene countenance of AP-1 besides PDL-1 in Hodgkin's lymphoma.

CONCLUSION

EBV-infested Hodgkin lymphoma has a sophisticated equal of the countenance of AP1 that facilitated oncogenic conduits besides immune cadence by binding to PD-L1. decreased the level of these inhibitor molecules is related to better local disease control.

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