Evaluation of IL-1 and IL-10 Concentration Level in Study of Epstein Barr Virus that Detected in Patients Suffered from Stomach Cancer in Al Hilla City

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ABSTRACT

Objective: The current research was conducted to determine the concentration level of IL-1 and IL-10 in Patients Suffered from Stomach Cancer infection.

Methodology: Descriptive case-control study design, the present study was conducted on 75 patients (56 males and 19 females) attended to, AL-Margan Hospital and AL-Sader Hospital in Hilla city from September, to November 2019. The patients were diagnosed clinically by physicians, who suffered from Stomach Cancer. In addition to that, a control group of 75 apparently healthy individuals (59 males and 16 females) who were without any history of disease were included.

Results: Individuals with *EBV* infection were found to have positive association with Stomach Cancer (18.7) in age groups(48-39)years.

Evaluation of immune status among *EBV* patients infected with Stomach Cancer through measuring the study showed a significant increase in the level of IL-1 and IL-10 among the clinical study groups. The study showed a significant increase at (P-value = 0.001) in IL-1 concentration (16.8 ± 4.6) in the age group (<59) compared to the control group (4.1 ± 2.1).This study also found that the concentration of Interleukin 10 showed a significant increase (188.1 ± 154.1) at (P- value = 0.01) in the age group (49-53) years compared to the control group (6.2 ± 0.3).

BACKGROUND

Gastric cancer is one of the riskiest infections and the fifth reason of death among all type of cancer. the most thoughtful is Epstein-Barr virus (EBV), Epstein-Barr virus is one of the most glutinous diseases that cause gastric cancer, it known also human herpesvirus [1]. There are many studies have indicated potential linkage between *Epstein-Barr virus* and some clinical manifestations such as associated stomach cancer and It is a specialized subset of stomach cancer and responsible for various human lymphoid and epithelial malignancies [2]. *(EBV), Epstein-Barr virus* is infected stomach cancer, which was first reported in 1990 Currently, stomach cancer is the most frequent EBV-associated malignancy [3]. The interleukin that included in the present research was Interleukin-1 and IL-10. IL-1 is a type of biological signal cytokine that can improve the body's natural response to disease as part of the immune system and it acts by binding to the interleukin-1 receptor and play more specialized roles on certain cell subsets, basophil and mast cells, and macrophages, respectively [4]. Interleukin 10 has an effect on sustaining resting B cells and has a vital role in stimulating immunoglobulin production from activated B cell [4].

MATERIALS AND METHODS

Patients with EBV and Control groups

 One hundred fifty of respondents were chosen in this study included 75 patients (56 males and 19 females) attended to Hospital in Hilla city. Control group consists of 75 apparently healthy individuals (59 males and 16 females) without any history of disease were clinically considered as healthy control also included in this study. This control group was examined by ELISA All control group was asked to fill a questionnaire and all had no family history of disease.

Samples Collection

Blood Collection

Peripheral blood (4 cc) was obtained under aseptic conditions from each subject by a vein puncture using a disposable syringe. Blood samples were divided into two tubes for Immunological evaluation IL-1 and IL-10 by Sandwich ELISA, shipped on dry ice and kept at –20 ºC prior to analysis. serum was obtained by putting the blood samples in a clean dry plain plastic tube and was allowed to clot at 37 ºC for 30 minutes before centrifugation. The tubes centrifuged at 6000 rpm for 5 minutes for viral RNA, DNA isolation. The blood sample and serum were placed in a cool box and were then transferred to the laboratory to be kept at -20℃ and processed within 24h.

Immunological techniques:

 1-Bioassay Technology Human Interleukin 1 Alpha & Beta ELISA Kit

Assay Principle

 This kit is an Enzyme-Linked Immunosorbent Assay (ELISA).

2- Bioassay Technology Human Interleukin 10 ELISA Kit.

Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human IL-10 antibody.

Statistics analysis

 The data were analyzed using the IBM SPSS Statistics (version 21). The changes and differences were considered significant when the probability (P) value was less than 0.05 (P<0.05) and highly significant when the probability (P) was less than 0.001(P<0.001). The results

were expressed as (Mean ± SD), Paired t-test has been used for the comparison between the patients and control groups and pearson relation bar for correlation between parameters. Pearson's correlation coefficients (r) were calculated to estimate the correlation between parameters.

RESULTS AND DISCUSSION
Immunological Cytokine of EBV

Table 1: Distribution of Cytokine types of concentration in clinical study groups

Group Statistics									95% CI	
Cytokine type	Clinical group	Mean	Std. Deviation	t. Test value	Df	P-value	Mean Difference	Std. Error Difference	Lower	Upper
Con_IL1	Cases	13.34	8.96	7.345	148	.000	7.98287	1.08686	5.83510	10.13063
	Control	5.35	2.85							
Con_IL10	Cases	78.92	92.63	6.829	148	.000	73.05676	10.69790	51.91640	94.19711
	Control	5.86	1.86							

The finding of table (1) shows that the mean concentratio interleukins 1 and 10 for the case group were more than concentrations of the control group, however, there was h statistically significant between two clinical study groups for both interleukins.

Table 2: Mean distribution of Epstein Barr Virus and its relationship with cytokine types between clinical study groups

Study groups	Cytokine type	Virus Status	Mean	Std. Deviation	t. Test value	Df	P-value
Cases	IL1	Positive	10.08	11.07	2.256	73	0.02
		Negative	12.25	8.13			
	IL10	Positive	79.26	93.93	0.015	73	0.98
		Negative	78.84	93.11			
Control	IL1	Positive	5.63	2.67	0.220	73	0.82
		Negative	5.34	2.88			
	IL10	Positive	6.06	1.26	0.269	73	0.78
		Negative	5.85	1.71			

The results of table (2) reveal that the means were the diffe between positive and negative cases for both interleukins (10) for both clinical study groups (cases-Control). How statistically, there was a statistically significant difference between positive and negative cases for Epstein Barr Virus in the interleukin 1 mean concentrations only.

Table 3: Mean concentration of IL-1 ± SD according to age groups and Gender.

Clinical groups		Mean concentration ±SD IL-1 No. (Conc. Pg/ml)			
		Cases With E. P. V	Cases Without E. P. V	Control With E. P. V	Control Without E. P. V
Age group(years)	29-33	-	13.4±2.62	-	4.2±2.2
	34-38	16.3±16.18	11.6±9.73	6.8±4.4	6.3±3.8
	39-43	14.5±6.76	9.9±7.0	-	5.8±1.7
	44-48	17.9±11.21	11.9±8.6	-	4.7±2.2
	49-53	-	10.1±6.4	-	6.7±3.6
	54-58	24.1±15.8	14.7±10.5	-	3.7±1.7
	59 and more	-	16.8±4.6	-	4.1±2.1
*Levene test= 24.256 P- value= 0.001		ANOVA test=	6.587	df=3	0.002
Gender	Male	17.7±11.4	12.4±7.4	6.1±2.7	5.3±2.9
	Female	25.4±3.4	11.7±9.7	4.9±1.9	5.2±2.7
*Levene test= 3.18 P- value=0.001		ANOVA test =	15.30	Df=3	0.012

The results of Table (3) show that there were statist significant differences between means concentrations of between the clinical study groups according to age gr (F=6.587, df=3 and p-value = 0.002). In addition, there v statistically significant difference between males and fen among clinical study groups (F=15.30, df=3 and p-value = 0. The present study agrees with the result reported by [5]. present study result does not agree or reverse result that has recorded by [6]. The current finding was compatible with that recorded by [7].
Results of this study agree with results recorded byt[8]. The result was consistent with the finding by [9]. Interpretation may be due to an immune status that affected by the severity of infections or may be due to genetic variation in the simultaneous occurrence of viral infection and that similar to logical explanation reported by [10].

Table 4: Mean concentration of IL-10 ± SD according to age groups and Gender.

Clinical groups		Mean concentration ±SD IL-10 No. (Conc. Pg/ml)			
		Cases With E. P. V	Cases Without E. P. V	Control With E. P. V	Control Without E. P. V
Age group(years)	29-33	-	83.9±44.3	-	5.6±0.1
	34-38	33.3±33.1	79.6±147.5	5.8±0.3	5.8±2.2
	39-43	16.2±1.7	69.4±19.1	-	5.5±1.2
	44-48	64.7±34.5	58.1±46.7	-	5.8±1.6
	49-53	-	188.1±154.1	-	6.2±0.3
	54-58	198.5±149.1	84.1±106.8	-	6.6±1.8
	59 and more	-	68.9±43.5	-	4.8±0.7
*Levene test= 4.695 P- value= 0.01		ANOVA test=	6.784	Df=3	0.002
Gender	Male	84.2±95.8	84.1±100.1	6.5±0.8	6.0±1.7
	Female	36.0±29.3	66.2±74.8	5.9±2.4	9.4±0.9
*Levene test= 9.70 P- value= 0.001		ANOVA test =	7.46	Df= 3	0.041

The results of Table (4) show that there were statist significant differences between means concentrations of between the clinical study groups according to age gr (F=6.784, df=3 and p-value = 0.002). In addition, there v statistically significant difference between males and fer among clinical study groups (F=7.46, df=3 and p-value = 0.04 was found this result was contravention with that recorded b The present study found that result does not agree with recorded by [12]. Also, it was nearly similar to the result found k While agreeing with [14]., these results come not to agree wit collectively these findings justify the elevation of IL-10 tha reported in the present study groups.

CONCLUSION
1-EBV infection can increase the risk of developing stomach cancer, this is because mutations in cells infected with EBV can lead to cancerous changes.
2-EBV has a virulence gene (EBERs) which plays a vital role in the pathogenicity of this virus, where induce the expression of interleukin (IL)-10 in BL cells, insulin-like growth factor (IGF)-1 in gastric carcinoma.
3- IL-10 highly increase in IBS patients with EBV and have a significant effect on the outcome of stomach cancer. Anyway, production of cytokines response plays a causal role in the development or progression disease.

RECOMMENDATIONS
1-More attention must be given for detection of EBV in blood of patients with gastric cancer.
2- Expanding the future study for re-evaluation the role of IL-1 in the immune response against Blastocystosis according to different infection that cause stomach cancer.
3. It was recommended to study the immunological relationship of IL-10 with other pathogens or systemic disease.

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