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RESEARCH ARTICLE

THE IMPACT OF CagA POSITIVE *HELICOBACTER PYLORI* INFECTION ON BCL-2 EXPRESSION IN GASTRIC MUCOSAL CELLS.

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Abstract

More than 50% of *Helicobacter pylori* strains produce the toxin CagA which introduced to inside the gastric mucosal cells. CagA induce up-regulation of p53 expression whereas, it plays as pro-apoptotic factor. Bcl-2 protein is one of the most important members of the family of anti-apoptosis, hence we aimed to investigate the association between CagA and Bcl-2. Paraffin embedded sections of gastric tissue from thirty patients had been included in this study. In addition, 10 apparently healthy volunteers as a control group. Procedure of In Situ Hybridization was carried out to detect the CagA cytotoxin and immunohistochemistry to evaluate the expression of Bcl-2. In patients group 24/30 cases of patients group gave a moderate staining, 5/30 cases gave substantial staining and only 1/30 cases gave slight staining. the immunoexpression of Bcl-2 in CagA negative cases (mean 44.706± 3.952 S.E.) was higher than that of CagA positive cases (mean 40.267± 1.476 S.E.). The CagA positive strain of *H. pylori* induce down-regulation of Bcl-2.

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Introduction:-

Since the discovery of the bacteria and designation as *Helicobacter pylori* in 1983 by Warren and Marshall (1984), the bacteria preoccupied the researchers in the field of microbiology as well as the clinical practice. *Helicobacter pylori* possesses a tremendous genomic material (Tomb *et al.,* 1997) and capabilities to colonized in the acidic environment of the stomach, thereby causing several types of stomach illness ranging from gastritis and atrophic gastritis leading to gastric cancer (Thung*et al.,* 2016). Bacterial large genome illustrated by multitude of virulence factors which possess complex paths in the pathogenesis, one of the most important virulence factor is the bacterial protein called cytotoxin associated gene A (CagA) antigen (Eftang*et al.,* 2012; Thung*et al.,* 2016). *Helicobacter pylori* strains can be divided into two major subpopulations depending upon their ability to produce the toxin CagA (Covacci*et al.,*1993; Tummuru*et al.,*1993, Akopyants*et al.,*1998). Approximately 50-70% of *H. pylori* strains isolated carry cag PAI (Xiang *et al.,* 1995). The cag PAI DNA segment encodes 31 proteins (Covacci*et al.,* 1999), CagA is the most immunogenic proteins of *H. pylori* (Olfat, 2003).

Apoptosis is very necessary process plays an important role in the formation of multicellular organisms and in organizing and maintaining the types of cells in the tissues under normal physiological and pathological conditions,

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the process involves specific milestones and a series of steps that eventually lead to local self-destruct (Forro 2009; Rathmell and Thompson, 2002).

There are two main apoptotic pathways: the extrinsic and the intrinsic, in which a large number of proteins play a role in these pathways either pro-apoptotic or anti-apoptotic (Elmore 2007). Bcl-2 protein is one of the most important members of the family of anti-apoptosis, works to prevent mitochondria path (intrinsic) of apoptosis and interact with other members of the family (Gryko*et al.,* 2014). In previously published work (Abood *et al.,* 2016) we investigated the association between CagA and p53 as pro-apoptotic protein. Hereby, we study the association of CagA positivity with Bcl-2 protein.

Materials and methods:-

A total of thirty patients had been included in this study according to the exclusion criteria enlisted in published work (Mohammed and Abood 2010; Abood *et al.,* 2016), in addition, demography, clinical presentation and groups were mentioned in the aforesaid works

Paraffin embedded sections of gastric tissue were cut into 4-5 µm thickness, mounted onto positively charged slides (superfrost /plus, Fisher brand ; U.S. Pat. 4481246) and drained the slides by fluffless blotting papers and left overnight to dry at room temperature (Divjak*et al.,* 2002).

Procedure of In Situ Hybridization was carried out as previously published (Mohamed and Abood 2010) to detect the CagA cytotoxin. On the other hand, immunohistochemistry (IHC) had been used to evaluate the expression of Bcl-2. The procedure of IHC was preformed according to manufacturer's instruction, using monoclonal mouse Anti-bcl-2 oncoprotein (DakoCytomation: Clone/REF: - N1587. Class/subclass: - IgG1, Kappa. IgG ready to use: - 0.05 mol/L) and Immunohistochemistry detection kit DakoCytomation LSAB2 System- HRP (Code KO673 DakoCytomation, USA).

The expression of Bcl-2 protein was quantified by counting the number of positive cells with brown (DAB) cytoplasmic staining under light microscopy X40. For the evaluation of Bcl-2 expression, a semi quantitative evaluation system was used to get the measure of the number of positive cells. Bcl2 expression patterns were graded according to the classification of Xia *et al.,* (2002) as shown in table 1.

Table 1:- TheSemi quantitative scoring system for Bcl-2 Immunostaining (Xia et al., 2002)

Bcl-2	***Score***	***Staining analysis***	***Stained cells (%)***
Negative	***0***	***0***	***<5***
Positive	***1***	***Slight***	***5-30***
	2	***Moderate***	***31-50***
	3	***Substantial***	***>50***

The data were analyzed statistically depending on the nature of the character (Snedecor and Cochran 1981) and data processing was done by using windows version of Statistical Package of Social Science (SPSS) version 16.

Results:-

The Cag PAI was detected by I.S.H. in 21 (70.0%) patients out of 30 and 9 (30.0%) gave negative results (Mohamed and Abood 2010).The gastric epithelial cells that show brown cytoplasmic staining consider as positive cells. In patients group 24/30 cases of patients group gave a moderate staining, 5/30 cases gave substantial staining and only 1/30 cases gave slight staining. Whereas in control group 8/10 cases gave substantial staining and 2/10 cases gave moderate staining. For further information, see figure (1).

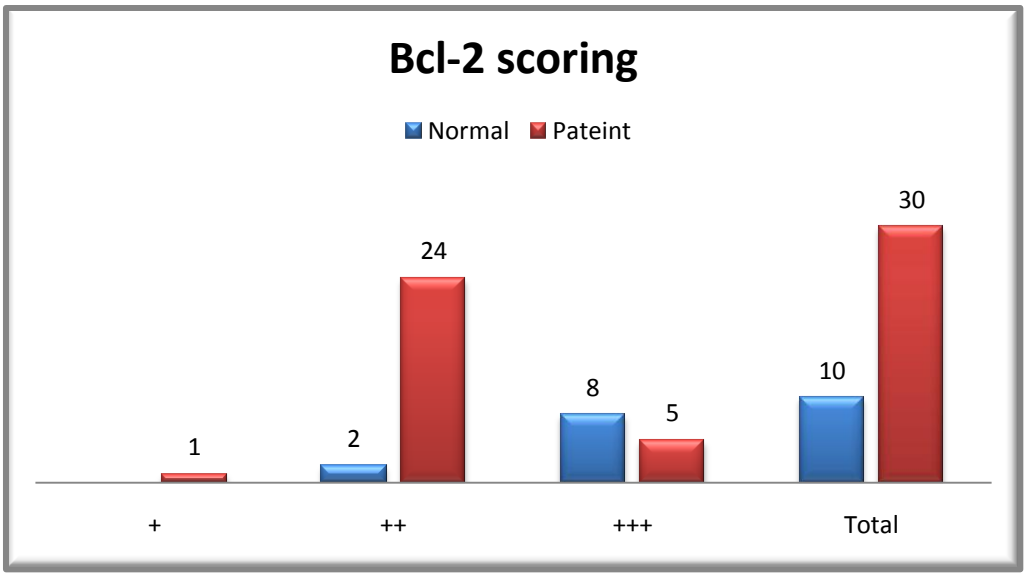

Figure 1:- Bcl-2 scoring in control and patients groups.

Depending on these results and by making simple comparison between control and patients groups using independent sample t-test we found that there is a high significant differences ($p \leq 0.001$) in Bcl-2 expression as shown in table (2).

Table 2:- independent sample t-test comparison between control and patients groups for Bcl-2.

Marker	group	No.	Mean	Std. Deviation	Std. Error Mean	T-Test p value
Bcl-2	Control	10	54.147	10.056	3.180	≤0.001
	Patient	30	41.598	8.638	1.577	

An independent sample t-test was performed to find out the differences in expression percentage of Bcl-2 among patients group depending upon CagA positivity in gastric tissue sections. We found out that there is no significant differences (p = 0. 202) in Bcl-2 expression between CagA positive and CagA negative patients, see table (3) for further information.

Table 3:- independent sample t-test comparison between CagA positive and CagA negative patients for Bcl-2.

Marker	Cag-A positivity	N	Mean	Std. Deviation	Std. Error Mean	T-Test p value
Bcl-2	Negative	9	44.706	11.856	3.952	0.202
	Positive	21	40.267	6.765	1.476	

Discussion:-

The Bcl-2 belonged to a large family of proteins that involved in apoptosis mechanisms which called Bcl-2 family. In general, this family is subdivided into pro-apoptotic (e.g. Bax, Bak, and Bad) and anti-apoptotic (e.g., Bcl-2, Mcl-1, and Bcl-XL) depending on these proteins functions (Favaloro*et al.*, 2012). Belka, andBudach(2002) found that Bcl-2 could counteract all actions of radiation to induce Bax or comparable pro-apoptotic proteins at the level of the mitochondria. Interestingly Bcl-2 was also shown to block apoptosis induction in response to the direct injection of cytochrome-c into cells, suggesting that Bcl-2 also acts downstream of the released cytochrome-c. Thomenius*et al.*,(2003), Nutt *et al.*,(2002), and Scorrano et al,. (2003) suggested that Bcl-2 can prevent activation of Bax localized to mitochondria and that Bcl-2 could control Bax activation through an intermediate.

We used immunohistochemistry technique to detect the expression of Bcl-2 proteins in gastric tissue infected by *H. pylori*. With regarding the CagA positivity, the results revealed that there is no significant differences (p=0.202) in Bcl-2 expression between CagA positive and CagA negative patients. And there is negative linear relationship

(Pearson correlation Coefficient=-0.240, p=0.202) between CagA positivity and Bcl-2 expression. In addition, the immunoexpression of Bcl-2 in CagA negative cases (mean 44.706± 3.952 S.E.) was higher than that of CagA positive cases (mean 40.267± 1.476 S.E.). These results suggesting that the CagA positive strain of *H. pylori* induce down-regulation of Bcl-2.

Our findings are in agreement with in vivo observations that *H. pylori* infection induces apoptosis associated with an up-regulation of Bax and down-regulation of Bcl-2 (Konturek*et al.,* 1999; Shibayama*et al.,* 2001; Cho, *et al.*,2015).The results of the present study come in agreement with the finding of Yang *et al.,* (2003), Liu *et al.*,(2005) and Eftang*et al.,* (2012) studies which stated that *H. pylori* induce apoptosis in the gastric epithelium via down regulation of the anti-apoptotic Bcl-2. and we have agreement with Cabral *et al.*, (2006)immunohistochemistry study in which they revealed that Bax and Bak expression was higher than Bcl-2 and Bcl-x, and was significantly higher in patients infected by *H. pylori* CagA positive strains than in those infected by negative strains.This study come in concordance with Ashktorab*et al.,*(2008) study in which they stated that *H. pylori*-induced apoptosis is associated with accumulation of mutated p53 protein and a decrease in Bcl-2.

Our immunohistochemical study for expression of Bcl-2 does not provide enough details about the dysfunction of the protein and/or gene mutation.The expression of Bcl-2 protein may be associated with the early events of the carcinogenesis before other oncogenic events such as p53 mutation take place (Anagnostopoulos*et al*., 2005).

Conclusions:-

The CagA positive *H. pylori* strain induce down-regulation of Bcl-2, and this favor to the pro-apoptotic pathway.

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