Immunohistochemical detection of TNF-α and IL-4 in chronic calculus cholecystitis

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Abstract

Gallstones are one of the most prevalent digestive disorders. Cholecystitis is inflammation of the gallbladder. The occurrence of the chronic cholecystitis often after several attacks of the acute cholecystitis and occurs usually due to the accumulation of the gallstones. Sometimes the chronic cholecystitis was without clinical signs, or have acute form. Pro-inflammatory cytokines (TNF-α) are vital cytokines that result in acute the inflammation of the gallbladder which leading to the production of the gallstone, however, IL-4 protects gallstone production.

Method: the current study was conducted on 95 patients referred to the surgical clinic of Al-Husain General Hospital, Al Nasiriyyah. Gallbladder tissue sample put in formalin then transport to the laboratory for immunohistochemical (IHC) examination the expression status of TNF-α and IL-4 in the patients with cholelithiasis as associated with (CCC).

Out of the 95 patient’s immunoexpression of TNF-α was high while IL-4 had low expression in CCC.

Conclusion: IHC expression of TNF-α was A more obvious as compared with IL-4 in CCC cholecystitis suggest that cytokines have a great and direct role in the cholelithiasis pathogenesis.

Keywords: proinflammatory cytokines, cholecystitis, cholelithiasis, immunohistochemistry, an anti-inflammatory cytokine.

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Introduction:

Gallstones are one of the widely occurring digestive disorders. Cholecystitis is the gallbladder inflammation. It may be continued for several months or results in repeated infection of the gallbladder, it is called chronic cholecystitis. Gallstone occurrence is a complicated development due to several environmental and genetic factors. Until recently, the role of the immune system in the pathogenesis of gallstones was not considered a valid topic of research interest¹. Cytokines are small, low molecular weights and non-structural proteins, it has a complex influence on the immunity and the inflammation. Cytokines are important indicators for the disease and the health cases therefore it is used for diagnostic and therapeutic purposes.² Cytokines are produced by the immune cells and produced by other cells related to the infection during cellular damage.³ Cytokines are commonly classified in one or the other category like tumor necrosis factor-alpha (TNF-α) as pro-inflammatory cytokines whereas interleukin-4 (IL4), recognized as anti-inflammatory cytokines.⁴ IL-4 is produced by TH2 cells, basophils, mast cells, and eosinophil. IL-4 is a stimulus TH2 cell and induces (IgE) in B-lymphocyte.⁵

Tumor necrosis factor-alpha is a cytokine that has an important role in the infection, apoptosis, and inflammation. tumor necrosis factor-alpha has two roles, the first role is the management of immune reaction as a mediator, the first role stimuli inflammatory response wherever it works as an immunosuppressive mediator and prevents tumorigenesis and autoimmune diseases.⁶ The previous studies revealed that serum levels of cytokines (IL1, TNF-α & IL-6) were significantly increased in gallstone patients sera comparing anti-inflammatory cytokine IL-4 concentrations in gallstone patients sera showed significant reduced compared to healthy controls.⁷ (IL-6) and (TNF-α) are primary cytokines results in increasing the gallbladder inflammation and increase of the gallstone
production, however, IL-4 protects gallstone production and some gallbladder diseases\(^9\). In the unresolved inflammation results in malignancy. TNF-\(\alpha\) is one inflammatory mediator produced in the carcinogenesis and the chronic inflammatory\(^\text{10}\). Deficiency of the IL-4 results in an increase in the gallstones production in the diet with high cholate, saturated fat, and cholesterol\(^\text{11}\).

**Materials and Methods**

Ninety-five consecutive samples after cholecystectomy were collected from Al-Hussain Teaching Hospital in Nasiriyah, Iraq.

**Ethical approval**

Given by the Medical Research at AL Nasiriya, Health Directorate ethical committee before the collection of the samples. A special form of a questionnaire constructed to gather data reviewed & revised by subject matter expert for testing the validity & enrichment.

**Immunohistochemistry staining procedure**

The Histopathology and immunohistochemical staining were performed in a private laboratory in Al- -Najaf.

**The monoclonal antibodies used in the immunohistochemistry staining procedure**

**IL4 antibody:** Anti-IL4 antibody is a Mouse monoclonal to IL4 provided 100μg, was dilutes 1:100. Direct from Abcam.

**TNF alpha antibody:** The Anti-TNF alpha antibody is Rabbit polyclonal to TNF alpha provided 100μg, was dilutes 1:100. Direct from Abcam.

**Statistical analysis**

Statistical analysis was done using Graph Pad prism. The results were expressed as mean ± standard deviations (mean ± SD). P-value <0.05 was considered significant. One-way ANOVA-Tukey's Test, one-way ANOVA-test and T-test was used to compare parameters in different studied groups. P ≤ 0.05 was used as the level of significance.

**Result**

**Immunohistochemistry study**

**Hematoxylin and Eosin Staining**

![Image](image_url)

**Figure(1):** Histological features of H and E under a microscope within power 4x showing chronic calculus cholecystitis the gallbladder mucosa is acutely inflamed with many inflammatory cells in the epithelium and lamina propria.
Figure (2): histological features under a microscope within power 10x showing chronic calculus cholecystitis include a variable inflammatory cell within muscularis and ulcerated in some areas, with hemorrhage and fibrosis, with foamy cytoplasm.

Figure (3): histological features under a microscope within 10x showing chronic cholecystitis include a variable inflammatory cell within both lamina propria and muscularis and hemorrhage with foamy cell.

Immunohistochemistry expression of TNF-α and IL-4:
TNF-α was detected with high expression in a tissue sample of CCC (49.5%) while IL-4 had low expression in CCC (17.9%).

Figure (4): A, B, C-an expression of the Tumor necrosis factor-alpha of gallbladder mucosa epithelial cells in chronic calculous cholecystitis cases (A) TNF-α expressions under power 4x (B) TNF-α expression on power 10x mostly within the epithelium of gallbladder(C) TNF-α expressions within gallbladder tissue on power 10x.
Figure (5): A-IL4 expression within gallbladder tissue under power 4x . B-gallbladder tissue with protein expression for (A) under 10x. C-IL4 expression in the mucosal layer of patients with chronic calculous cholecystitis.

Figure (6): Distribution of TNF-α among calculus cholecystitis patients

Figure (7): Distribution of IL-4 among calculus cholecystitis patients

Correlation between TNF-α and IL-4:
There is no marked Correlation between and IL-4 and TNF-α among CCC patients.
Discussion

Immunohistochemistry study

Immunohistochemistry expression of TNF-α and IL-4

TNF-α was detected with high expression in a tissue sample of CCC (49.5%) while IL-4 had low expression in CCC (17.9%). A previous study showed that TNF-a was detected in all CCC patients and play a role in gallstone pathogenesis[8]. TNF-α is the main cytokines leading to an increase of the gallbladder inflammation and results in gallstone formation, but, IL-4 protects gallstone production and some gallbladder illness cases. TNF-α is a cytokine and has a great role in the host defense against the parasite, virus, and bacterial infections. TNF-α has great importance during the infection, but hyper-production could be harmful[12].

Correlation between TNF-α and IL-4

The significant correlation between TNF-α and IL-4 in CCC are none. When protein expression of proinflammatory cytokines (TNF alpha) higher than anti-inflammatory cytokine (IL4) this indicated the presence of immune predisposition in patients for gallstone. IL-4 could protect gallstone production, while TNF-α results increase in the gallbladder inflammation which leading to gallstone formation[9]. TNF alpha high expression than IL4 levels of proinflammatory cytokines (IL1, TNF-α & IL-6) were significantly increased in gallstone patients sera, While anti-inflammatory cytokine IL-4 concentrations in gallstone patients sera showed significant reduced[8].

Conclusions:

1. The elevation of pro-inflammatory cytokine (TNF-alpha) expression reflects the presence of inflammation associated with gallstones.
2. The reduction of anti-inflammatory cytokine (IL4) expression reflects the protective role in gallstone patients.

Reference:

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