

## The Prognostic Impact of Heat Shock Protein-70, IL-23 and IL-17A Levels in Colorectal Cancer

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### Abstract

**Background:** Cytokines play a pivotal role in the induction of host immune responses against tumor growth carcinoma and are involved in the development and progression of colorectal cancer in humans. Thus, the aim of the study was to determine Heat shock protein-70 (HSP-70), IL-23 and IL-17A levels in the development and progression of colorectal (CRC) cancer.

**Methods:** A group of 40 patients and 20 healthy volunteers were included in the study. The quantitative determination of serum HSP-70, IL-23 and IL-17A were performed by ELISA.

**Results:** A significant levels of HSP-70, IL-23 and IL-17A in CRC patients compared to the controls was recorded and the highest level was detected in patients with stage D, which was significantly greater than those in stages A, B, and C.

**Conclusions:** HSP-70 and IL-23 levels were significantly elevated in patients' sera, in contrast to IL-17A, In addition, increased levels were strongly associated with the progression of colorectal (CRC) and these serum cytokine profiles may play a role in tumor development.

**Keywords:** Colorectal, HSP70, th17, IL-23 and tumor progression.

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### I. Introduction

Heat shock proteins (HSPs) are intracellular, evolutionary conserved proteins with a most important role in maintaining homeostasis of the cells by holding and folding other proteins as well as by protecting the genetic information. Heat shock proteins are usually divided into families according to their molecular weight; currently, 10 kD (HSP10), 27 kD (HSP27), 40 kD (HSP40), 60 kD (HSP60), 70 kD (HSP70), 90 kD (HSP90), and 110 kD (HSP110) heat shock protein families are known. All these HSPs- HSP27, HSP70, and HSP60 primarily play essential, but diverse roles in tumorigenesis and metastasis formation, by promoting autonomous cell proliferation and inhibiting programmed cell death [1]. Interleukin-23 is a heterodimeric cytokine (p40/p19) consisting of a novel p19 subunit and the p40 subunit of IL-12p70. Studies have defined IL-12p70 as an important factor for the differentiation of naive T cells into IFN- $\gamma$  producing Th1 cells and exhibits anti-tumor activity [2], [3], [4] and [5] memory T cells to proliferate and produce IFN- $\gamma$ . In addition, IL-23 induces the production of a proinflammatory cytokine, IL-17, from activated T cells, namely Th17 [6]. IL-17A is one of six members (A-F) of the IL-17 family [7]. IL-17 is produced by Th17 cells and other cells: CD8<sup>+</sup> T cells,  $\gamma\delta$ T cells, invariant NKT cells, mast cells, and granulocytes [8]. Moreover, IL-17 has been shown to promote angiogenesis and regulate production of a variety of proangiogenic factors, including vascular endothelial growth factor [9] and [10] IL-17 expression is increased in inflammatory bowel disease [11], and tumor-infiltrating Th17 cells are found in human colorectal cancer and are associated with shortened disease-free survival [12] and [13]. Studies with mouse models have also revealed a role for IL-17 signaling in the development of colorectal tumors [14].

### II. Subjects and Methods

#### Subjects

The present study was conducted from 2012 to 2014 on 40 Iraqi CRC patients were collected from Baghdad Medical City Teaching Hospital (16 females and 24 males; mean age  $51.4 \pm 17.10$  years, ranged between (21-81 years) at different stages. In addition to 20 apparently healthy individuals considered as controls.

#### Estimation of Cytokine level

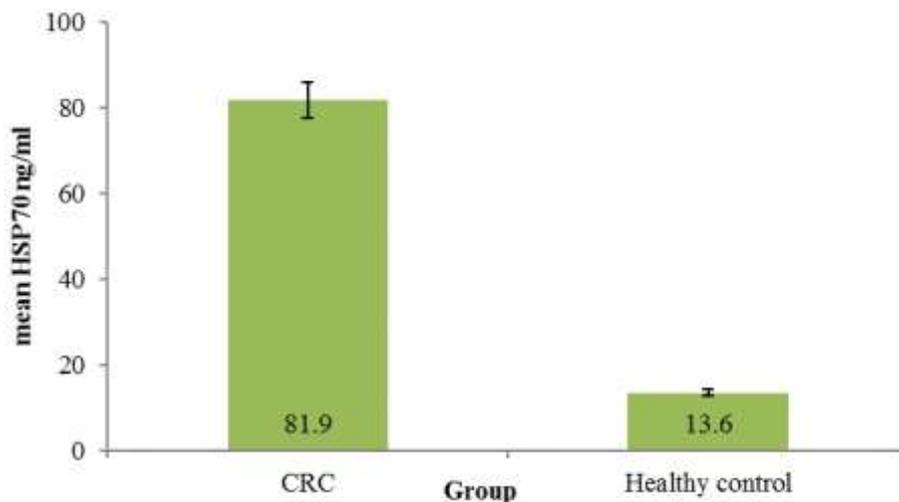
Enzyme linked immunosorbent assay (ELISA) was used to estimate HSP-70, IL-23 and IL-17A by ELISA kit according to the instructions provided by manufacturer (Quantikine, R&D Systems).

**Statistical Analysis**

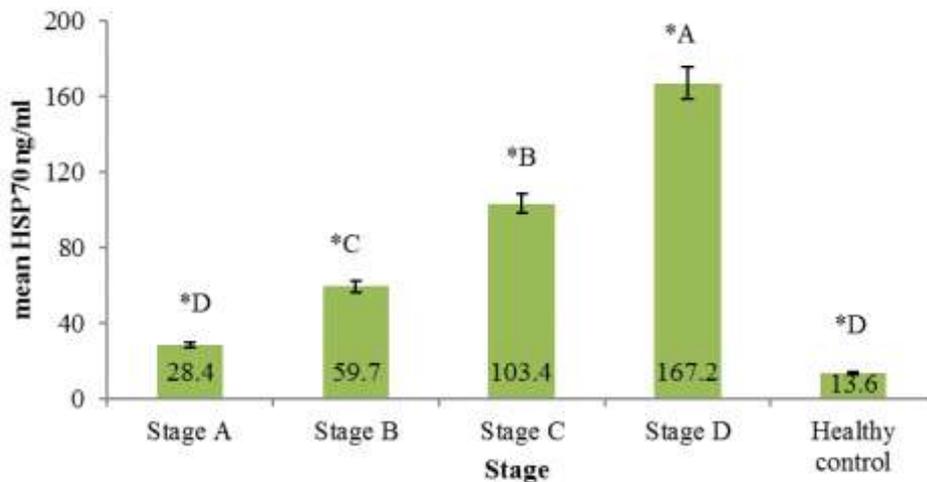
Differences in HSP70, IL-23 and IL-17 patients with different stages of CRC and healthy donors were analyzed using SPSS program version 20. Results were expressed using simple statistical parameters such as mean and standard error. Differences between means were assessed by ANOVA, followed by either LSD or Duncan test. Acceptable level of significance was considered to be  $P \leq 0.05$ .

**III. Results**

Results revealed a significant elevation in serum HSP70 level among CRC patients ( $81.9 \pm 9.5$  ng/ml) in comparison to that of healthy control ( $13.6 \pm 1.6$  ng/ml) ( $p \leq 0.05$ ) (Figure 1A), Since results showed the highest level was recorded in stage D ( $167.2 \pm 14.9$  ng/ml) followed by stage A, B, and C ( $28.4 \pm 4.4$ ,  $59.7 \pm 3.8$  and  $103.4 \pm 9.9$  ng/ml) respectively (Figure 2A). Also, the current study revealed positive linear correlation ( $r=0.869$ ) between serum HSP70 level and the progression of the disease (Table 1).



**Figure (1A): Serum HSP70 in CRC and healthy control**



**Figure (2A): Serum HSP70 in CRC stages and healthy control**

\*: Significant differences  $P \leq 0.05$  (Duncan test)

The results of IL-23 are clearly shown in (Figure 1B), an interesting significant increase of mean serum level in CRC patients was recorded ( $68.2 \pm 8.3$  pg/ml) as compared to healthy control ( $10.2 \pm 1.3$  pg/ml). Concerning the correlation of serum IL-23 level with Duke's classification of tumor, revealed that higher concentration was recorded in patients with stages C and D ( $102.7 \pm 14.4$  and  $116.7 \pm 15.1$  pg/ml) respectively followed by stage A and B ( $19.8 \pm 2.5$  and  $33.6 \pm 4.8$  pg/ml) respectively (Figure 2B). Moreover, positive correlation between serum IL-23 level and stages ( $r=0.758$ ), ( $P \leq 0.01$ ) were recorded (Table 1).

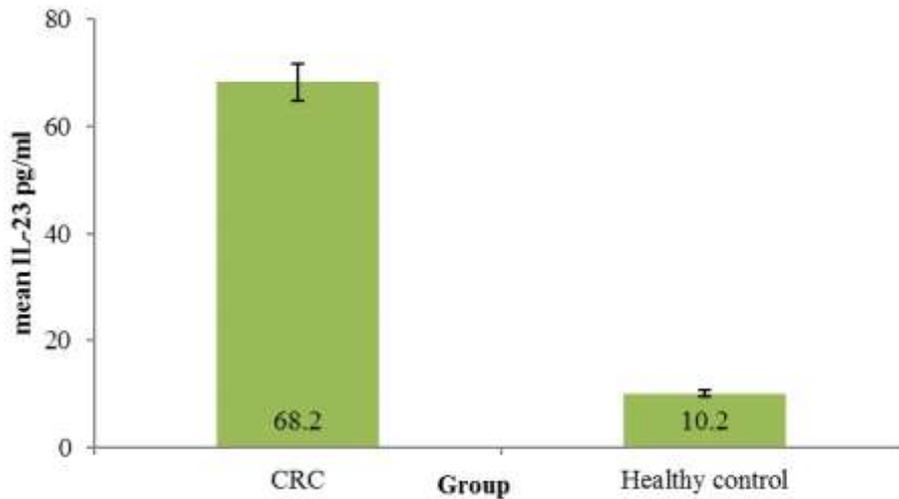


Figure (1B): Serum IL-23 in CRC and healthy control

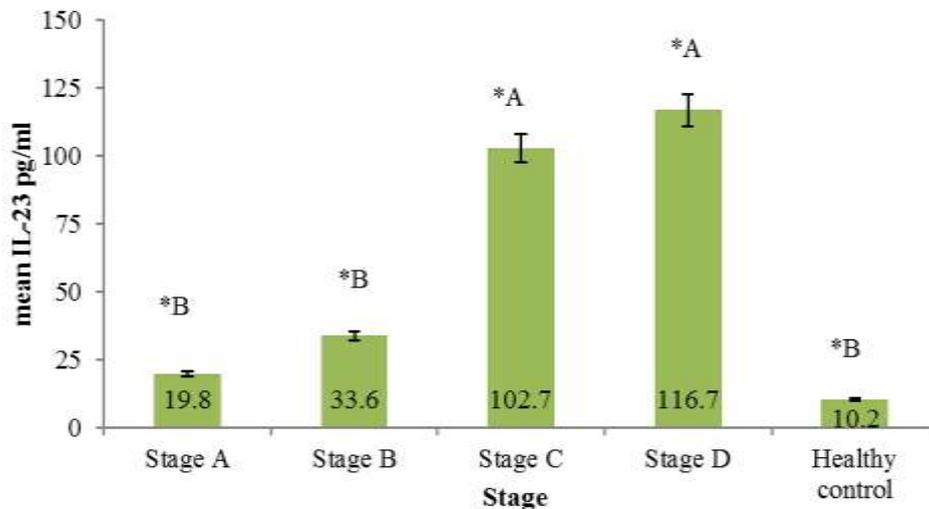


Figure (2B): Serum IL-23 in CRC stages and healthy control  
 \*: Significant different between means at  $P \leq 0.05$  (Duncan test)

Results showed IL-17A was significantly higher in CRC than control ( $48.0 \pm 4.3$  vs.  $5.5 \pm 0.4$  pg/ml), (Figure 1C). CRC patients with stage D displayed significantly higher level of IL-17A ( $76.6 \pm 8.6$  pg/ml) followed by stage C ( $51.9 \pm 5.4$  pg/ml) than those with A and B ( $21.5 \pm 4.2$  and  $42.0 \pm 5.0$  pg/ml) respectively (Figure 2C). A positive significant correlation was recorded between IL-17A and stages ( $r=0.729$ ), ( $P \leq 0.01$ ) (Table 1).

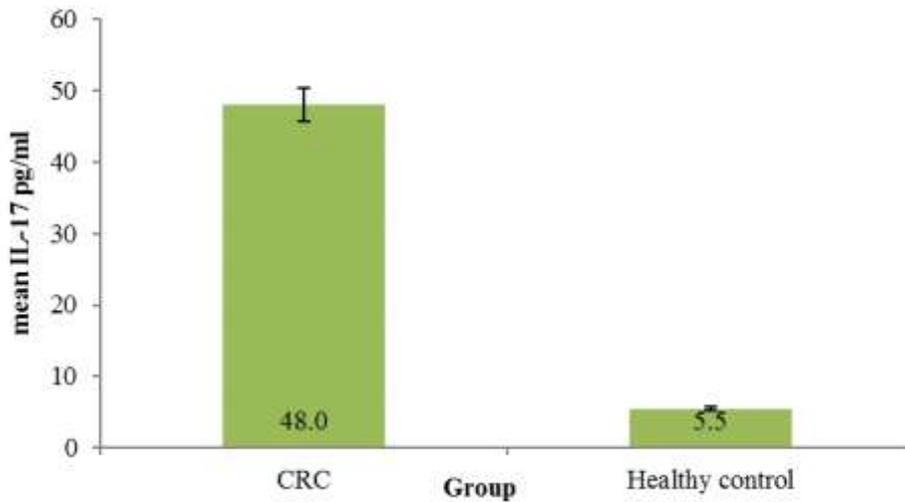


Figure (1C): Serum IL-17 in CRC and healthy control

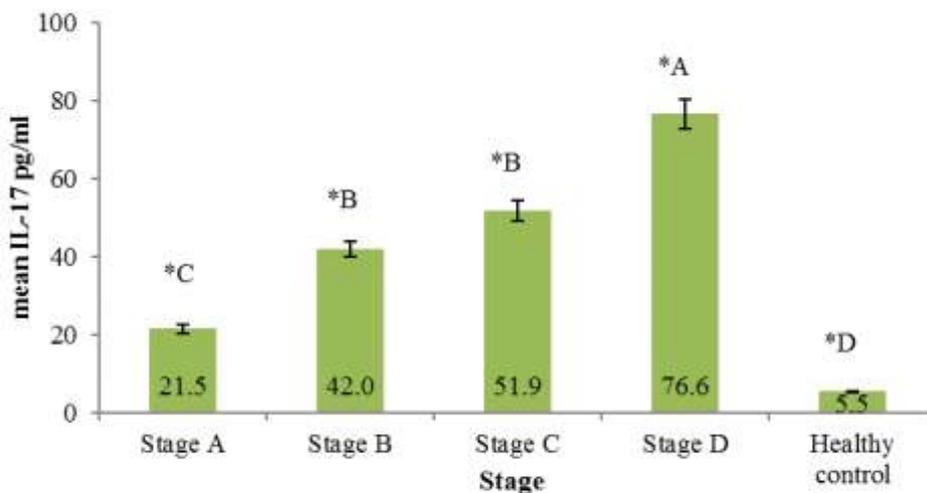


Figure (2C): Serum IL-17 in CRC stages and healthy control  
\*:Significant differences between means at  $P \leq 0.05$  (Duncan test)

Table (1): Correlation between HSP-70, IL-23 and IL-17A and Stages in patients with CRC

		HSP-70	IL-23	IL-17A
Stage	Pearson correlation (r)	0.869**	0.758**	0.729**

\*\*Correlation is significant at the level 0.01 (2-tailed)

#### IV. Discussion

Over the past few years, evidence has demonstrated that interleukins carry out important functions in tumor development, cell differentiation, inflammation and metastasis. Results showed that high level of serum HSP-70 in patients with CRC in related with tumor progression came in accordance with reports have documented that HSP70 reduces or blocks caspase activation and suppresses mitochondrial damage and nuclear fragmentation [15]. These findings were supported by Li *et al.* [16] who found HSP70 inhibited apoptosis downstream of the release of cytochrome *c* and upstream of the activation of caspase-3. This antiapoptotic effect was explained by the HSP70-mediated modulation of the apoptosome. Indeed, HSP70 has been demonstrated to directly bind to apoptosis protease-activating factor-1 (Apaf-1), thereby preventing the recruitment of procaspase-9 to the apoptosome [17]. The ATPase domain of HSP70 has been described to be necessary for this interaction [18]. Other reports have shown that HSP70 interacts with procaspase-3 and procaspase-7 and prevents their maturation, thereby inhibiting the caspase-dependent apoptotic signaling [19]. Results demonstrated distinct differences in the serum level of pro-inflammatory cytokine IL-23 and IL-17A in CRC patients. Recent work on a similar subject investigated mechanisms responsible for tumor-elicited inflammation in a mouse model for colorectal tumorigenesis, which like human colorectal cancer exhibits up-regulation of IL-

IL-17A and IL-23 [20]. Langowski and colleagues [21], showed that IL-23 promotes tumor incidence and growth in various human cancers and plays a key role in chronic intestinal inflammation and its up-regulation in malignant tissues parallels augmented levels of the “metastatic biomarker” matrix metalloproteinase MMP-9, tumor necrosis factor TNF-alpha, and increased levels of angiogenesis [22],[23] and [24]. In this respect, IL-17A, which is largely produced by activated memory T lymphocytes, stimulates both innate immunity and host defense, and plays an active role in inflammatory diseases, autoimmune diseases, and cancer. More specifically, IL-17A induces the expression of nuclear factor-kappa B (NF-κB), chemokines CXCL8, CXCL6 and CXCL1, growth factors G-CSF, GM-CSF (granulocyte-macrophage colony-stimulating factor), IL-6, and adhesion molecules (ICAM-1), leading to augmented neutrophil accumulation, granulopoiesis, and inflammatory responses [25] and [26].

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