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Strong Positive Correlation Between IL-10 and CXCL-10 Methylation in Iraqi Hodgkin Lymphoma Patients

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Abstract

Background: The unique characteristic of Hodgkin Lymphoma (HL) is its intense inflammatory microenvironment and the ability to evade immune response through over-expression of immune-related genes (i.e., aberrant DNA methylation). Serum cytokines such as IL-10, CXCL-10 and IFNg are significantly elevated, yet there is limited knowledge regarding the association between serum cytokine methylation patterns and peripheral blood (PB) methylation status for these genes. **Methods:** This case-control study included 50 newly diagnosed HL patients and 50 matched healthy controls from Iraq. Serum levels of IL-10, CXCL-10 and IFN-γ were measured by ELISA, and methylation percentages of promoter regions of *IL-10*, *CXCL-10* and *IFNG* were assessed by quantitative methylation-specific PCR (qMSP). Correlation analyses evaluated associations between cytokines and their methylation status, as well as inter-gene methylation relationships. **Results:** Patients showed dramatically elevated median IL-10 (84.9 vs. 5.2 pg/mL), CXCL-10 (423.2 vs. 14.7 pg/mL) and IFN-γ (704.1 vs. 84.7 pg/mL) (all $p<0.0001$). Methylation of *CXCL-10* (64.4% vs. 52.3%, $p=0.040$)

and *IFNG* (90.2% vs. 27.0%, p<0.0001) was significantly higher in patients, while *IL-10* methylation did not differ between groups (p=0.190). Strikingly, a robust positive correlation was observed between *IL-10* methylation and *CXCL-10* methylation (Pearson r = 0.545, p < 0.001) in HL patients. No significant correlations were found between any cytokine level and its own methylation percentage. EBV status (78% seropositive) did not influence either cytokine levels or methylation values. **In conclusion,** there is a clear positive relationship with both IL-10 and CXCL 10 methylation, indicating a potential linkage of dysfunction of epigenetics within HL regardless of the EBV status. The dissociation of hypermethylation from the cytokine overexpressed within HL suggests that the local specific environment takes precedence over repressive DNA methylation marks. This study has provided new insights into immunopathogenesis in HL**.**

Keywords: Hodgkin lymphoma; DNA methylation; IL-10; CXCL 10; epigenetic correlation; Iraqi cohort.

Introduction

Classical Hodgkin lymphoma (cHL) is a B-cell neoplasm that is distinguished by a remarkable histological appearance: Hodgkin and Reed-Sternberg (HRS) malignant cells are distributed throughout an immense collection of inflammatory cells, including T-cells, macrophages, eosinophils, and plasma cells (Küppers, 2009; Connors et al., 2022). HRS cell interactions with the tumor microenvironment (TME) help produce a plethora of cytokines and chemokines that contribute to symptoms experienced throughout the body, as well as substantial suppression of the immune system (Aldinucci et al., 2010; Steidl et al., 2011). IL-10 is one of many mediators that have an immunosuppressive effect by inhibiting Th-1-type responses, and promoting the activity of regulatory T-cells, whereas IFN-γ and the IFN-γ-inducible chemokine CXCL-10 (IP-10) are abnormal in HL but do not produce

sufficient anti-tumor immune effects. (Saraiva & O'Garra, 2010; Liu et al., 2011; Gocher et al., 2022).

Along with soluble factors, epigenetic modifications (mainly via DNA methylation) ant the immune system gene expression in cancer (Baylin & Jones, 2016; Curtis & Sparrow, 2022).Promoter hypermethylation typically silences gene transcription; however, recent studies in haematological malignancies suggest that chronic inflammation can induce complex epigenetic changes that may not directly correlate with expression in peripheral blood cells (Portela & Esteller, 2010; Zhang et al., 2022). In HL, whether DNA methylation of cytokine genes in circulating leukocytes reflects the disease state or correlates with protein levels remains understudied. Moreover, the potential for coordinated epigenetic regulation across functionally distinct immune genes is unknown. The present study aimed to (i) measure serum IL-10, CXCL-10 and IFN-γ and the methylation status of their corresponding genes in Iraqi HL patients; (ii) test associations with EBV serostatus; and (iii) explore correlations among methylation levels of different cytokines, with a special focus on the relationship between *IL-10* and *CXCL-10* methylation.

Materials and Methods

Study design and participants

A total of 50 newly diagnosed classical Hodgkin lymphoma patients at AL-Diwaniyah Teaching Hospital, AL-Diwaniyah, Iraq, between December 2024 and November 2025. Fifty age- and sex-matched healthy volunteers without any history of cancer or chronic inflammatory disease served as controls. Exclusion criteria included prior chemotherapy, radiotherapy, active infection, or other malignancies. All participants provided written informed consent, and the study protocol was approved by the institutional ethics committee.

Blood collection and EBV status

Peripheral venous blood (5 mL) was collected in serum separator tubes and EDTA tubes. Serum was separated and stored at −80°C until cytokine analysis. EBV IgG antibodies were measured by ELISA (sunlong ,chain), and EBV DNA was quantified by real-time PCR targeting the *BamHI-W* region (promega, USA). Patients with both positive IgG and detectable DNA were classified as EBV-positive.

Cytokine quantification

Serum concentrations of IL-10, CXCL-10/IP-10, and IFN-γ were determined using high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (sunlong ,chain) according to the manufacturer's protocols. Samples were run in duplicate, and intra-assay coefficient of variation was <8%.

DNA extraction and methylation analysis

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using the Nucleic Acid Purfication Kit (China). Bisulfite conversion was performed with the MethylEdge TM Bisulfite Conversion System Kit (Promega,USA). Quantitative methylation-specific PCR (qMSP) was performed for promoter CpG islands of *IL-10*, *CXCL-10* and *IFNG* using validated primer/probe sets. Fully methylated and unmethylated controls were used to calculate the percentage of methylated reference (PMR). All assays were run in triplicate.

Statistical analysis

Data were analyzed using SPSS version 26.0 (IBM Corp.). Normality was assessed by Shapiro-Wilk test; non-parametric tests were used for skewed data (Mann-Whitney U test for group comparisons). Categorical variables were compared using chi-square test. Correlations between continuous variables were

evaluated using Pearson or Spearman correlation as appropriate. Receiver operating characteristic (ROC) analysis evaluated diagnostic accuracy. A two-tailed $p < 0.05$ was considered statistically significant.

Results

Demographic and EBV characteristics

The HL group comprised 27 (54.0%) males and 23 (46.0%) females (male: female ratio 1.17:1); controls included 30 (60.0%) males and 20 (40.0%) females (p=0.545). Mean age (±SD) was 44.2±14.6 years for patients and 45.6±13.9 years for controls (p=0.622). EBV seropositivity with confirmed qPCR was detected in 39/50 patients (78.0%), whereas 11 (22.0%) were EBV-negative.

Cytokine levels and diagnostic performance

HL patients exhibited markedly elevated serum levels of IL-10, CXCL-10 and IFN-γ compared to controls (all p<0.0001, Table 1). ROC analysis showed perfect discrimination for each cytokine: IL-10 cutoff 46.06 pg/mL (AUC=1.00, 100% sensitivity/specificity), CXCL-10 cutoff 226.0 pg/mL (AUC=1.00), and IFN-γ cutoff 454.3 pg/mL (AUC=1.00). No significant differences in cytokine levels were found between EBV-positive and EBV-negative patients (p>0.05).

Table 1. Serum cytokine concentrations in HL patients vs. healthy controls

Cytokine	Group	Median (pg/mL)	IQR	p-value
IL-10	Patients	84.921	5.711	<0.0001

Table 1. Serum cytokine concentrations in HL patients vs. healthy controls

Cytokine	Group	Median (pg/mL)	IQR	p-value
	Controls	5.187	2.855	
CXCL-10	Patients	423.238	47.944	<0.0001
	Controls	14.738	13.350	
IFN-γ	Patients	704.076	66.355	<0.0001
	Controls	84.660	74.937	

DNA methylation profiles

IL-10 methylation: Median percentage was higher in patients (88.83%) compared to controls (58.71%), but the difference was not statistically significant (p=0.190), with wide interquartile ranges indicating substantial heterogeneity. **CXCL-10 methylation**: Significantly higher in HL patients (64.37% vs. 52.25%, p=0.040). **IFN-γ methylation**: Highly significantly elevated in patients (90.20% vs. 26.96%, p<0.0001) (Table 2). EBV status did not influence methylation of any gene (all p>0.05).

Table 2. Methylation percentages (%) of IL-10, CXCL-10 and IFNG in patients and controls

Gene	Group	Median (%)	IQR	p-value
IL-10	Patients	88.831	146.61	0.190 (NS)
	Controls	58.710	217.54	
CXCL-10	Patients	64.373	39.04	0.040 (S)
	Controls	52.251	37.68	
IFN-γ	Patients	90.200	87.58	<0.0001 (HS)
	Controls	26.961	12.88	

Correlation analysis: methylation of IL-10 and CXCL-10

In the HL patient group, Pearson correlation revealed a strong positive relationship between *IL-10* methylation and *CXCL-10* methylation ($r = 0.545, p < 0.001$). In contrast, no significant correlations were found between serum IL-10 levels and *IL-10* methylation ($r = –0.136, p=0.347$), nor between CXCL-10 and its methylation ($r = –0.139, p=0.336$). IFN-γ methylation did not correlate with its own protein level ($r = –0.013, p=0.927$) nor with IL-10 or CXCL-10 methylation. No inter-cytokine correlations (IL-10 vs. CXCL-10, IL-10 vs. IFN-γ, CXCL-10 vs. IFN-γ) were significant. The full correlation matrix is presented in Table 3.

Table 3. Pearson correlation matrix between cytokines and methylation percentages in HL patients (n=50)

Variable	**IL-10 (protein)**	**CXCL-10 (protein)**	**IFN-γ (protein)**	**Methylation IL-10**	**Methylation CXCL-10**	**Methylation IFN-γ**
IL-10 (protein)	1	0.040	–0.019	–0.136	0.004	0.029
CXCL-10 (protein)	0.040	1	–0.102	–0.028	–0.139	0.024
IFN-γ (protein)	–0.019	–0.102	1	0.034	0.213	–0.013
Methylation IL-10	–0.136	–0.028	0.034	1	**0.545****	0.115
Methylation CXCL-10	0.004	–0.139	0.213	0.545**	1	-0.033
Methylation IFN-γ	0.029	0.024	–0.013	0.115	–0.033	1

*** p < 0.001; All other p-values >0.05. No significant correlations between protein levels and corresponding methylation.*

Discussion

This study provides the first evidence in an Iraqi HL cohort of a strong positive correlation between promoter methylation of *IL-10* and *CXCL-10* (r=0.545, p<0.001), indicating coordinated epigenetic changes across two functionally divergent immune genes. Although the overexpression of IL-10 and CXCL-10 exists in both adaptive Immunity (Immunosuppression) and non-adaptive Immunity (Pro inflammatory), this dysregulation occurs systemically throughout Hodgkin Lymphoma, with increases in the levels of methylated peripheral blood cells corresponding to one another. This finding suggests that there is an epigenetic regulatory mechanism that encompasses the two proteins and may be influenced by shared inflammatory stimuli; these stimuli may be mediated by the effects of chronic IFN gamma or the activation of NF kappa B, resulting in global alterations in DNA methyltransferase or TET enzyme activity (Curtis & Sparrow, 2022;Zhang et al., 2022). Chronic inflammation, being a characteristic of HL, is considered to induce"epigenetic drift" in leukocytes and the co-methylation of IL-10 and CXCL-10 may be indicative of a chronic immune stimulation footprint (Portela & Esteller, 2010). The evidence supporting the idea that high levels of cytokine proteins have no relationship to their own methylation indicates that, in HL, the strong transcriptional signals from the tumour micro-environment (for example, STAT3 or NF -kappa B), can counteract the repressive effects of DNA methylation marks present in those cell types capable of secreting cytokines (Kube et al., 2001; Weniger & Küppers, 2021). Our data support others who have found that there is a lack of relationship between methylation and expression in several inflammatory disease processes (Wilson et al., 2007; Smale et al., 2014).

The strong find positive correlation between IL-10 and CXCL-10 methylation does not translate or correlate to IFN γ methylation, suggesting that epigenetic regulation of IFNG might use different transcription factors or have different target cells (Schoenborn et al., 2007). The lack of differences in methylation and protein levels between EBV+ and EBV- HL indicates that the observed epigenetically programmed immune milieu is a common feature of HL biology within our cohort and are consistent with data demonstrating convergent pathway activation, irrespective of viral status (Carey et al., 2017; Carbone & Gloghini, 2018). The extraordinary diagnostic value of IL 10, CXCL 10 and IFN γ (AUC=1.00) suggests they will serve as useful adjunctive serological biomarkers, specifically when tissue diagnosis is difficult.

IL-10 and CXCL-10 are both highly co-methylated at the individual level, indicating they have the potential to be used as an epigenetic signature (composite) of immune dysregulation/disease burden, but further study is required to determine if these methylation levels revert after successful treatment. Limitations of this study include a moderate sample size, the inability to sort PBMCs for cell type–specific analysis, and the absence of follow-up data for determining prognostic correlation with epigenetic alterations. Our data provide novel pathways to understand how the HL microenvironment causes co-ordinated epigenetic alteration that may not directly result in protein level changes but are likely to be stable markers of disease.

There is a significant relationship between IL-10 and CXCL-10 promoter methylation in Iraqi patients who suffer from Hodgkin's lymphoma, indicating coordinated epigenetic dysregulation. The increased levels of serum cytokines, despite hypermethylation of CXCL-10 and IFNG, suggests an influence of microenvironmental signals that can overcome the repression of methylation markers. This information contributes to the knowledge of immunopathogenesis

associated with HL and indicates that development of diagnostic and monitoring using multi marker epigenetic and cytokine panels have potential value in HL.

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