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RESEARCH ARTICLE

Chemotherapy upregulates gene expression of IL-1 and Prostaglandin in the blood of patients with non-Hodgkin's lymphoma

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Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt

ABSTRACT

Background: Most lymphoma patients, including non-Hodgkin lymphoma (NHL), show dysfunction in immunity, especially after chemotherapy, resulting in a lack of responses to treatment and tumor relapses. This dysfunction could be due to the high expression of inflammatory mediators, especially prostaglandin, including its different types and critical cytokines such as IL-1. **Aim**: This pilot study aimed to measure gene profiling of IL-1 and PG pathways in the peripheral blood of NHL patients. This study was conducted on 3 healthy volunteers and 9 NHL patients; before, during, and after induction of chemotherapy CHOP (chemotherapy regimen consists of Cyclophosphamide, Hydroxydaunorubicin, Oncovin and Prednisone). The patients were recruited from Tanta Cancer Centre, Tanta. Total RNA (mRNA) was extracted, and then IL-1 (NFKB-1, PELI-1, IL-1α, CCL-2, and CASP-1), Prostaglandin (PTGS-2, PTGER-2, and EDN-1) pathways were analyzed by Gene Chip RNA HTA 2.0 Arrays (Affymetrix). Real-time PCR (qRT-PCR) was used as validation of the genes of the IL-1 and PG pathways. **Results**: Microarray analysis showed that gene expression of IL-1 pathways, including NFKB1, PELI-1 and CCL2, was downregulated at early diagnosis as compared to healthy control; their expression was upregulated during as well as after chemotherapy. CASP-1 and IL-1α gene expression, however, was downregulated in the early-diagnosed group and then upregulated after chemotherapy. PTGS-2, PTGER2 and EDN1 in the prostaglandin pathway were downregulated in early-diagnosed patients and then, upregulated during chemotherapy as well as after chemotherapy. **Conclusion:** Chemotherapy regulates the expression of certain genes of the IL-1 and Prostaglandin pathways. These inflammatory mediators may be represented as biomarkers in diagnoses and prognosis.

Keywords: Array, Affymetrix, Chemotherapy, Gene expression, IL-1, Lymphoma, Microarray, Prostaglandin

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Correspondence to

Sohail M. Khalil, PhD
Department of Zoology,
Faculty of Science,
Tanta University, Egypt
Tel.: 01062306664
Email: sohailagalal37@gmail.com

INTRODUCTION

Malignant lymphoma is a proliferative process of the lymphopoietic portion of the reticuloendothelial system, involving cells of either the lymphocytic or histiocytic series in varying degrees of differentiation, which occurs in an effectively homogeneous population of a single cell type (Perez-Ruiz et al., 2013). Non-Hodgkin Lymphomas (NHL) Non-Hodgkin lymphomas (NHLs) constitute a heterogeneous group of lymphoproliferative malignancies characterized by diverse biological behaviors and less predictable clinical courses compared to Hodgkin lymphomas and have a far greater preference to disseminate to extra-nodal locations. Nearly 25% of NHL cases who arise in extra have a far greater preference to disseminate to extra nodal locations. Nearly 25% of NHL cases arise in extra nodal locations and most of them seem to involve both nodal and extra nodal sites (Singh et al., 2020). IL-1 is a master cytokine of systemic inflammation, which plays an important role in tumor progression. IL-1 is subject to regulation by components of the IL-1 and IL 1 receptors (ILR) (Garlanda et al., 2013). Negative regulators include a decoy receptor (IL-1R2), receptor antagonists (IL-1Ra), IL-1R8, and anti-inflammatory (IL-37) (Supino et al., 2022).

IL-1 acts at different levels in tumor initiation and progression, including tumor angiogenesis, activation of the IL-17 pathway, induction of myeloid-derived suppressor cells (MDSC) and macrophage recruitment, invasion and metastasis. (Mantovani et al., 2018). IL-1β inflammatory mediator produced mainly by activated monocytes/macrophages, possess a wide variety of multiple overlapping activities in the areas of inflammation and immunology. These cytokines can induce prostaglandin biosynthesis in several types of cells (Kaneko et al., 2019). Prostaglandins have been linked to inflammation, female reproductive cycle, vasodilation, or bronchodilator/ bronchoconstriction. (Jara- Gutierrez and Baladron 2021).

PGE2 regulates fever, kidney function, pain, mucosal integrity, blood vessel homeostasis, and inflammation. It is produced by cancerous stromal cells and enhances tumor cell proliferation and survival, promotes angiogenesis, and induces metastasis. During tumor progression, PGE2 exerts its activity through ligation with four E-type proteinoid (EP) receptors 1–4 (EP 1–4), by acting on releasing cells and neighboring cells (Finetti et al., 2020).

Cyclooxygenase (COX) plays a significant role in the conversion of arachidonic acid to prostaglandin. It has two isoforms, COX-1 and COX-2. COX-1 maintains the homeostatic level of prostaglandin and COX-2 is induced by mitogenic or inflammatory stimuli, including cytokines, growth factors and tumor promoters (Attiq et al., 2018). It participates in carcinogenesis and cancer progression. COX-2 functions through activating carcinogens, inhibiting apoptosis, promoting angiogenesis, modulating immunological responses, and influencing tumor invasion by activation of matrix metalloproteinases (Szweda et al., 2019). The connection between COX-2 and tumor lymphangiogenesis has been discovered in breast, gastric, prostate and lung cancer (Ma et al., 2012). Our problem is NHL exhibits immune dysfunction, especially after chemotherapy, leading to poor therapeutic response and tumor recurrence. Dysfunction may be due to high especially various types of prostaglandins and key cytokines such as IL-1. IL-1 is a major inflammatory cytokine that plays an important role in tumor progression and can induce prostaglandin biosynthesis in several cell types associated with inflammation and cancer.

Dysfunction may be due to high especially various types of prostaglandins and key cytokines such as IL-1. IL-1 is a major inflammatory cytokine that plays an important role in tumor progression and can induce prostaglandin biosynthesis in several cell types associated with inflammation and cancer. We hypothesize that alteration of PG and IL-1 signaling pathways in the presence of chemotherapy can be used as prognostic markers. This study aimed to analyze the gene array profiling of IL-1 and PG pathways in the peripheral blood of NHL patients.

SUBJECTS AND METHODS

Subjects

This pilot study was conducted on nine patients with NHL (5 males and 4 females); the patients and the three healthy female volunteers were of the same age, between 40 and 70 years old, and of different sexes. The patients were grouped into three groups (Early diagnosed, during chemotherapy, and after chemotherapy. The patients were recruited from Tanta Cancer Center, Egypt. The Ethical Committee approved the research study: The approval code is 3012/01/15, Faculty of Medicine, Tanta University, Egypt, before the commencement of the study.

Collection of blood samples

Five ml of whole venous peripheral blood was collected from control subjects and patients. Samples were put in ethylenediamine tetraacetic acid (EDTA) tubes. The anticoagulated blood was diluted with an equal volume of PBS, and the diluted blood was slowly layered over the same volume of Ficoll-Hypaque solution and then centrifuged for 40 min at 400-×g, at 22°C. Leukocyte cells were finally suspended in PBS and stored at −80°C for RNA extraction.

RNA extraction

Total RNA (mRNA) fractions were extracted from the whole blood samples using TRIzol (Invitrogen) in combination with RNeasy Mini Kit from Qiagen, (USA) according to the manufacturer's protocol. The method combines phenol/guanidine-based lysis and silicon membrane-based purification of total RNA.

Microarray gene expression profiling

Single-stranded cDNA was synthesized from total RNA isolated from blood cells. A hybridization cocktail was prepared at room temperature, and the appropriate amount of hybridization master mix (Fragmented and Labeled ss-DNA, Control Oligo B2, 20X Hybridization Controls, 2X Hybridization Mix, DMSO, Nuclease-free Water) was added to each fragmented and biotin-labeled ss-cDNA sample to prepare hybridization cocktail.

Table 1. Forward and Reverse Sequences of selected genes (most up and down regulated genes)

Gene name	Forward Sequence	Reverse Sequence
NFKB1	GCAGCACTACTTCTTGACCACC	TCTGCTCCTGAGCATTGACGTC
PELI1	TGTAGTAACTGACACGGTTCCT	TCCATCTGATGTCTTCCATTTGG
CASP-1	CCACAGACCTTCCAGGAGAATG	GTGCAGTTCAGTGATCGTACAGG
IL-1A	TGTATGTGACTGCCCAAGATGAAG	AGAGGAGGTTGGTCTCACTACC
CCL2	AGAATCACCAGCAGCAAGTGTCC	TCCTGAACCCACTTCTGCTTGG
EDN1	CTACTTCTGCCACCTGGACATC	TCACGGTCTGTTGCCTTTGTGG
PTGS2	CGGTGAAACTCTGGCTAGACAG	GCAAACCGTAGATGCTCAGGGA
PTGER2	GACCACCTCATTCTCCTGGCTA	AACCTAAGAGCTTGGAGGTCCC

The hybridization cocktail reaction was then incubated for 5 min at 99°C, then for 5 min at 45°C in a thermal cycler using the hybridization cocktail program. It is then hybridized into the probe array during 16-hour incubation. The whole-genome cDNA-mediated annealing, selection, and ligation (WG-DASL) were performed. The whole genome HTA DASL assay was performed following the manufacturer's instructions using 200 ng RNA. Samples are tested based on low-quality of RNA concentration by nanodrop. Arrays were washed to remove nonspecifically bound nucleic acids and stained on Fluidics Station 450. Chips then scanned on Gene-Chip Scanner 3000 7G system (Affymetrix®). The microarray expression levels were normalized with two controls: exogenous and internal controls. Internal control of the samples included human 5.8s rRNA (gi555853) ''ten identical probes". Tag and hybridized to the miRNA array. When total RNA is titrated, the average signal of these probes is also titrating. Microarray raw data was analyzed by using the Oligo package (v1.48.0) on R (v3.6.1). In terms of differences in probe intensity distributions, the quality control, which included background correction, normalization, and calculating expression, was performed on the raw intensities of each array. Files were analyzed with Genotyping console for quality control analysis. Expression Console (version 1.3.1) and Transcriptome analysis console (TAC Version 3.0) were used as analysis software. We identified IL-1 and prostaglandin pathways as inflammatory mediators for the most dysregulated genes (up and downregulated genes) and validated it by qRT-PCR.

Quantitative real-time PCR (qRT-PCR)

For validation of the Gene expression of prostaglandin and IL-1, purification of RNA was evaluated by nanodrop and then, cDNA synthesis was performed. Samples with purity ranging from 1.8 to 2.0 were included in the cDNA synthesis protocol. For the detection of gene expressions, cDNA was prepared in the step of reverse transcription reaction. The cDNA acted as a template for the qRT-PCR technique by using miScript, HiSpec Buffer, a miScript Primer Assay (forward primer) and the miScript SYBR Green PCR Kit (Qiagen), which includes the miScript Universal Primer (reverse primer) and QuantiTect SYBR Green PCR Master Mix.

Statistical analysis

All data are the means of three replicates. The normality of the data was tested with the Kolmogorov-Smirnov test. One–way analysis of variance (ANOVA) was applied to determine the significant differences among different groups.

If there is a significant difference between means, Tukey post hoc comparisons among different groups were performed. For all statistical tests, P values <0.05 were considered statistically significant.

RESULTS

NFKB-1 is upregulated during and after chemotherapy in NHL patients

As regards the gene expression of NFKB1 (one of IL-1 pathway genes) by gene array analysis (Figure 1A), a decrease in the expression in lymphoma patients by 33.91-fold regardless of the treatment, as compared to the health control. Its expression showed increases during chemotherapy and after chemotherapy by 10.8- and 3.53-fold, respectively, as compared to early diagnosis patients (P-value <0.001). Validation of the gene expression using qRT-PCR showed similar trends to gene array analysis. It was found that the fold change of NFKB-1 expression in the early-diagnosed group was downregulated by 8-fold as compared to the healthy control. However, the gene expressions during the chemotherapy group and after chemotherapy were upregulated by 2, 1.4-fold, respectively, as compared to the early diagnosed group (P Value <0.001) (Figure 1B).

PELI1 is upregulated during and after chemotherapy in NHL patients

As regards the gene expression of PELI1 (one of IL-1 pathway genes) by gene array analysis (Figure 2A), a decrease in the expression was observed in lymphoma patients by 316.42-fold regardless of the treatment as compared to the healthy control. Its expression showed increases during chemotherapy and after chemotherapy groups of 76.15, 1.4-fold, respectively, as compared to the early-diagnosed group. patients (P-Value <0.001). Validation of the gene expression using qRT-PCR showed a similar trend to that of gene array analysis. It was found that the fold change of PELI1 expression in the early-diagnosed group was downregulated by a 14-fold change compared to the healthy control. However, the gene expressions during the chemotherapy group and after chemotherapy were upregulated by 2, 6.1-folds, respectively, as compared to the early-diagnosed group (P-Value <0.001) (Figure 2B).

CASP-1 is upregulated after chemotherapy

As regards the gene expression of CASP1 (one of IL-1 pathway genes) by gene array analysis (Figure 3A), a decrease in the expression in lymphoma patients by 6.76-fold change regardless of the treatment as compared to the healthy control. Its expression showed increases during chemotherapy and after chemotherapy groups by -1.91, -11.67-fold, respectively, as compared to early–diagnosed patients (P-Value <0.001).

Validation of gene expression using qRT-PCR showed that the fold change in CASP1 expression in the early diagnosed group was downregulated by 2-fold compared to the healthy control. However, gene expressions in the chemotherapy group and after chemotherapy were downregulated by 6 and 2.2-fold, respectively, compared to the early diagnosed group (P-value < 0.001) (Figure 3B).

Upregulation of IL-1α after chemotherapy

As regards gene expression of IL-1α (one of IL-1 pathway genes) by gene array analysis (Figure 4A), it was found to decrease in the expression in lymphoma patients by 30.31-fold change, regardless of the treatment, as compared to the healthy control. Its expression showed a slight decrease during chemotherapy by 1.21-fold and showed a slight increase after chemotherapy group by -1.4-fold as compared to early-diagnosed patients (P-Value <0.001). Validation of the gene expression using qRTPCR, it was found that the fold change of IL-1α expression in the early-diagnosed group was downregulated by 25-fold as compared to healthy control. However, the gene expression during the chemotherapy group and after chemotherapy was up-regulated by 4.25, 30.7-folds, respectively, as compared to the early-diagnosed group (P-Value <0.001) (Figure 4B).

CCL2 is upregulated during and after Chemotherapy

As regards gene expression of CCL2 (one of IL-1 pathway genes) by gene array analysis (Figure 5A), an increase in the expression in lymphoma patients by -64- fold change, regardless of treatment, as compared to control. Its expression showed increases during chemotherapy and after chemotherapy groups by -45.3, 13.25-fold, respectively, as compared to early-diagnosed patients (P-value <0.001).

Validation of gene expression using qRT-PCR showed that the fold change in CCL2 expression in the early-diagnosed group was downregulated by 1-fold compared to the healthy control. The gene expression during the chemotherapy group was up-regulated as compared to the early-diagnosed group by 2.5- foldchange. Its expression after chemotherapy group was downregulated by 1.8-fold as compared to the early diagnosed group (P-value <0.001) (Figure 5B).

EDN1 is upregulated during and after chemotherapy

As regards gene expression of EDN1 (one of the Prostaglandin pathway genes) by gene array analysis (Figure 6A), a decrease in the expression was observed in lymphoma patients by 10.9-fold change regardless of the treatment, as compared to the healthy control.

Its expression showed increases during chemotherapy by 1.3-fold and showed decreases after chemotherapy groups by 1.6-fold as compared to early-diagnosed patients (P-value <0.002). Validation of the gene expression using qRT-PCR.

It was found that the fold change of EDN1 expression in the early-diagnosed group was downregulated by 3-fold as compared to healthy control. Its expression during the chemotherapy and after chemotherapy groups were upregulated by 1.6, 6-fold, respectively, as compared to the early-diagnosed group (P-Value <0.001) (Figure 6B).

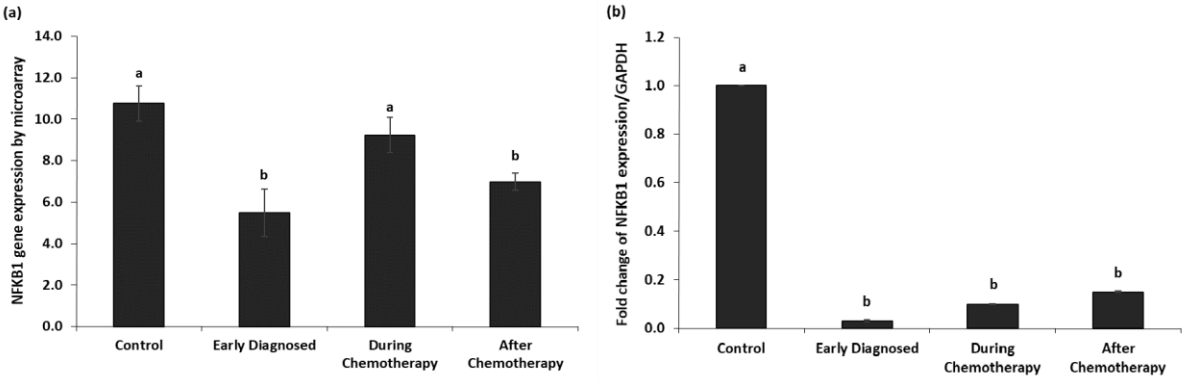

Figure 1. Expression gene of NFKB-1 (IL-1 pathway gene) in lymphoma in early-diagnosed group, during chemotherapy and after chemotherapy groups: A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

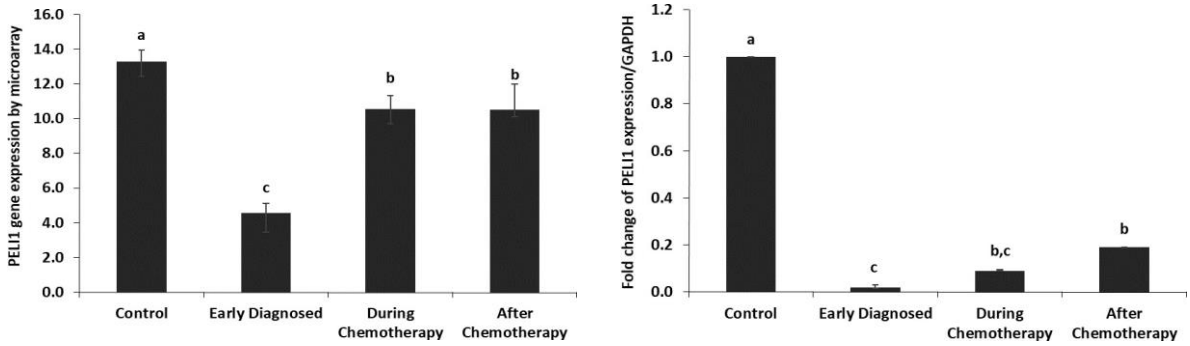

Figure 2. Shows Expression gene PELI1 (IL-1 pathway gene) in lymphoma in early diagnosed group, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

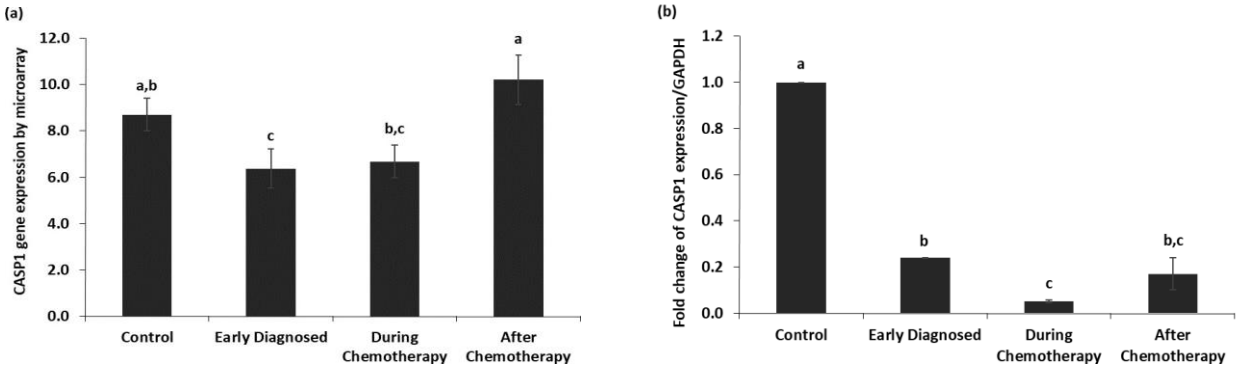

Figure 3. Shows expression gene CASP1 (IL-1 pathway gene) in lymphoma in early diagnosed group, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

PTGES-2 is upregulated during and after chemotherapy in NHL patients

As regards gene expressions of PTGS2 (one of Prostaglandin pathway genes) by gene array analysis (Figure 7A) it was found a decrease in the expression in lymphoma patients by 10.96-fold regardless of the treatment as compared to the healthy control. Its expression showed increases during chemotherapy and after chemotherapy groups by 1.3, 1.6-fold, respectively, as compared to early-diagnosed patients, (P-Value <0.001). Validation of the gene expression using qRT-PCR showed a similar trend in gene array analysis. It was found that the fold change of PTGS2 expression in the early-diagnosed group was downregulated by 2.7-fold as compared to the healthy control. However, the gene expressions during the chemotherapy group and after chemotherapy were upregulated by 1.6, 2.6- folds, respectively, as compared to the early-diagnosed group, (P-Value <0.001) (Fig. 7B).

PTGER-2 upregulated during chemotherapy

As regards the gene expression of PTGER2 (one of Prostaglandin pathway genes) by gene array analysis (Figure 8A), a decrease in the expression in lymphoma patients by 921-fold change, regardless of the treatment compared to the healthy control. Its expression showed increases during chemotherapy and after chemotherapy groups by 824.3, and 650.4-fold, respectively, as compared to early-diagnosed patients (P-Value <0.003). Validation of the gene expression using qRT-PCR showed a similar trend in gene array analysis. It was found that the fold change of PTGER2 expression in the early-diagnosed group was downregulated by 3.7-fold as compared to the healthy control. However, the gene expression during the chemotherapy group and after chemotherapy were upregulated by 2.2, 2.6- folds, respectively, as compared to the early diagnosed group, (P-Value <0.001) (Figure 8B).

IL-1β and PGE2 levels peak during chemotherapy and then decline

The concentration level of IL-1β in the healthy control group was 329.7 pg/mL, serving as the baseline reference. In the early diagnosed lymphoma group, IL-1β decreased slightly to 314 pg/mL, corresponding to a 1.04-fold change relative to control. During chemotherapy, IL-1β showed a marked increase to 403 pg/mL, representing a 1.22-fold elevation compared to control, indicating a strong proinflammatory response associated with treatment. After chemotherapy, IL-1β concentration declined to 307 pg/mL, which reflects a 1.07-fold change compared to control, suggesting suppression below baseline levels. Interestingly, these findings demonstrate that IL-1β levels transiently rise during chemotherapy but subsequently decrease to lower values than those observed in healthy individuals (P-Value = 0.051) as shown in (Fig .9A).

The concentration of PGE2 in the healthy control group was 362.5 pg/mL and was taken as the baseline reference. In the early diagnosed lymphoma group, PGE2 was 363.5 pg/mL, corresponding to a fold change of ~1.00 compared to control, indicating no meaningful difference. During chemotherapy, PGE2 rose to 401.5 pg/mL, representing a 1.11-fold increase relative to control, suggesting a chemotherapy-associated elevation in PGE2 production. After chemotherapy, the concentration decreased to 353.5 pg/mL, which reflects a 1-fold change compared to control, showing a reduction below baseline. Importantly, this post-chemotherapy decrease was statistically significant compared to the early diagnosed group (P = 0.006).

Overall, these findings indicate that PGE2 levels remain stable in early diagnosis, increase during chemotherapy, and decline after treatment to value lower than both control and early diagnosed groups as shown in (Fig. 9B). Correlation analysis between IL-1β and PGE2 concentrations across different patient groups revealed a consistent positive relationship. In the early diagnosed group, both markers showed nearly parallel concentrations with a modest correlation, suggesting an early inflammatory response. During chemotherapy, the correlation was strongest, as elevated IL-1β levels were closely mirrored by increased PGE2 concentrations, indicating that treatment-induced inflammation may simultaneously drive both cytokine and prostaglandin pathways. After chemotherapy, the levels of both IL-1β and PGE2 declined, yet their correlation remained evident, reflecting a

coordinated decrease in inflammatory mediators following treatment. These findings suggest that IL-1β and PGE2 are dynamically linked throughout disease progression and treatment, with the strongest association observed during chemotherapy as shown in Figure 9C.

DISCUSSION

Many studies reported that Lymphoma diagnosis and knowledge have been facilitated in recent years by the development of high throughput molecular tools, such as expression microarrays aimed at quantifying the expression of RNA or protein. To characterize this tumor more comprehensively, and to identify new diagnostic and prognostic markers (Kos et al., 2021). Few studies characterized expression profile of genes of inflammatory mediators, especially prostaglandin, including its different types and signaling pathways of critical cytokines such as IL-1, particularly in cancer patients. In this study, we determined the alteration of expression genes of IL-1 and prostaglandin pathways in early-diagnosed NHL, during and after induction of chemotherapy by microarray analytical method and confirmed it by qRT-PCR. Other studies reported that NF-κB pathways can facilitate the development of novel agents to treat malignancies and overcome drug resistance in patients with relapsed or refractory tumors (Balaji et al., 2018). This could illustrate our findings that the gene expression of NFκB-1 was upregulated during and after chemotherapy induction compared to early diagnosed NHL patients. Another study reported that PELI1 expression was high in cases of high-grade B-cell lymphoma such as diffuse large B-cell lymphoma, Burkitt lymphoma, and plasmablastic lymphoma (Choe et al., 2016). On the other hand, it was reported that PELI1 expression levels in patients with diffuse large B cell lymphomas (DLBCLs) were positively correlated with BCL6 expression, and PELI1 overexpression was closely associated with poor prognosis in DLBCLs (Park et al., 2014). Interestingly this could illustrate our findings that the gene expression of PELI1 was downregulated in early-diagnosed NHL patients compared to healthy control and upregulated during and after induction of chemotherapy compared to early-diagnosed NHL patients. Overexpression of IL-1α, particularly transient overexpression, may have anti-tumorigenic effects in lymphoma cells that have been induced to express IL-1α transiently (Baker et al., 2019). In addition, it was reported that many inflammatory cytokines, such as IL1α, were found in the tumor microenvironment and were strong predictors of overall survival in DLBCL patients (Zhao et al., 2016). In our study, we found that downregulation of IL-1α in early diagnosed. NHL patients as compared to healthy control and the upregulation of the gene expression after induction of chemotherapy as compared to the early-diagnosed group.

Caspases play a critical role in the regulation of apoptosis, cell differentiation, inflammation, and innate immunity, and several are mutated or have been altered expression in NHL. It was reported that CASP1 was significantly associated with all major NHL subtypes (Shalini et al., 2015). In our study, we found that the gene expression of CASP1 was downregulated in early-diagnosed NHL patients compared to healthy control, and it was upregulated after chemotherapy induction as compared to early-diagnosed groups. There are several reports that CCL2 was expressed in a variety of cancers and has been linked to poor prognosis. Inhibiting or blocking the CCL2/CCR2 signaling axis has thus become a focus of cancer therapy (Dasoveanu, Park et al. 2020). On the other hand, a previous study showed that CCL2 was found in high concentrations within the follicular lymphoma (FL)-cell niche.

It is also upregulated in mesenchymal stromal cells obtained from healthy age-matched donors (HD-MSCs) after co-culture with malignant B cells, and it is overexpressed in FL-MSCs. CCL2 expression was also higher in NHL than in reactive lymph nodes, and patients had a shorter survival time (Guilloton et al., 2012). Interestingly, we found the gene expression of CCL2 was downregulated in early-diagnosed NHL patients compared to the healthy control and was upregulated during the chemotherapy group as compared to early-diagnosed groups. Regulation of IL-1α in early diagnosed NHL patients compared to healthy controls.

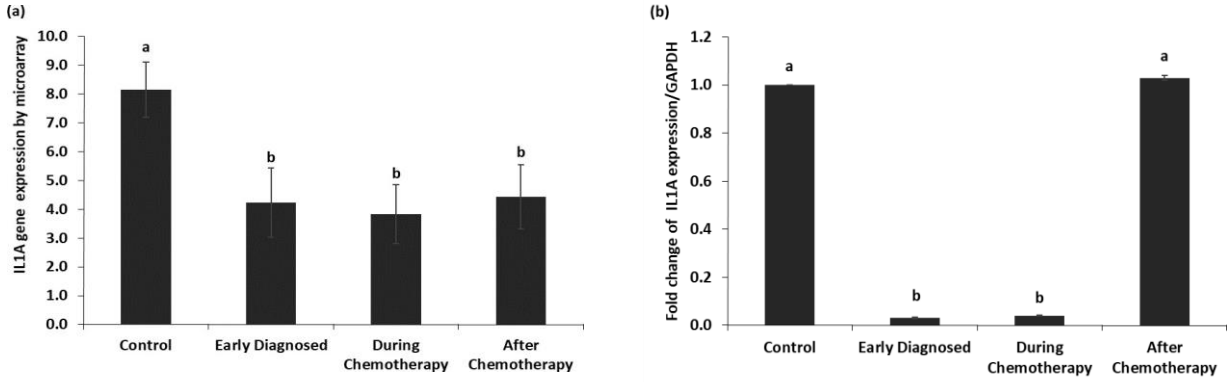

Figure 4. Expression gene of IL-1α (IL-1 pathway gene) in lymphoma in early-diagnosed group, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qrt-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

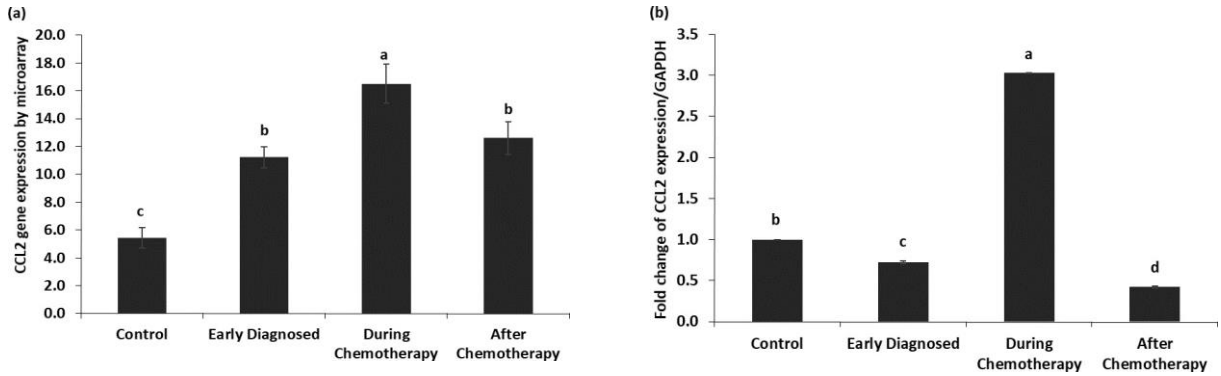

Figure 5. Expression gene of CCL2 (IL-1 pathways gene) in lymphoma in early-diagnosed groups, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

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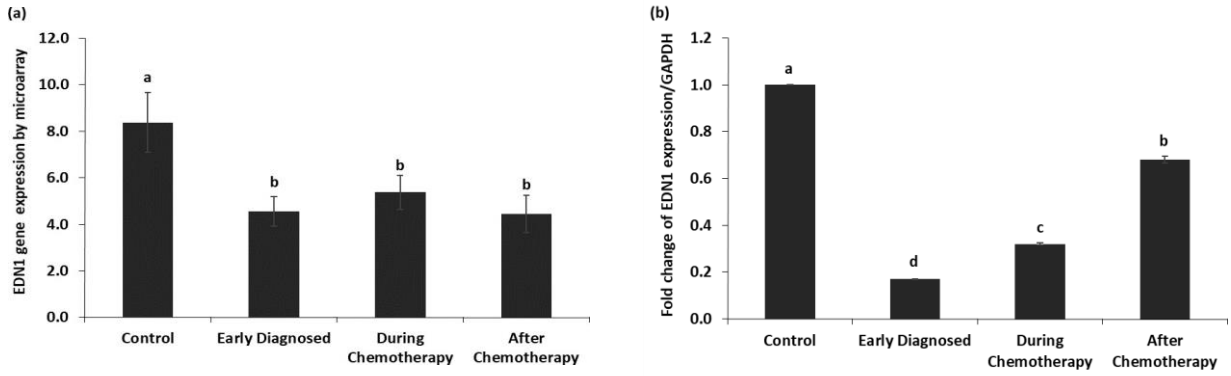

Figure 6. Expression gene of EDN1 (Prostaglandin pathway gene) in early diagnosed, during chemotherapy and after chemotherapy groups. A) Using Microarray. B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

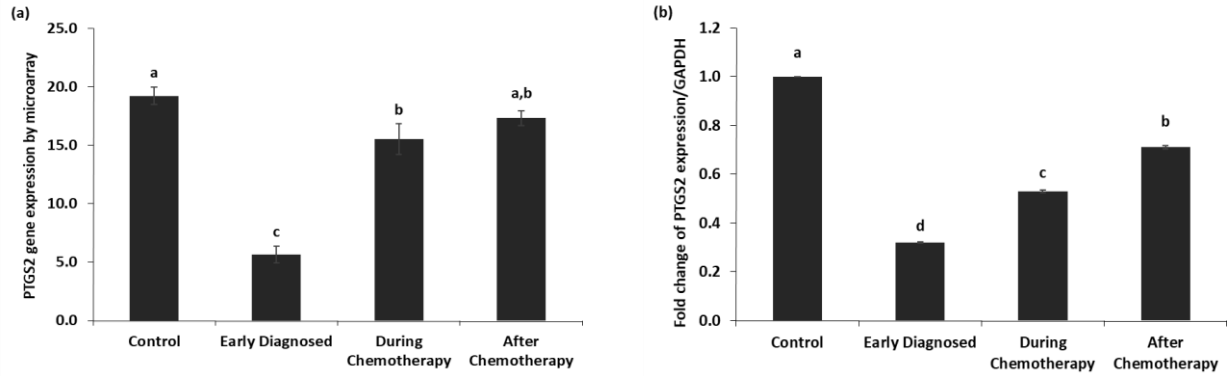

Figure 7. Expression gene of PTGS2 (Prostaglandin pathway gene) in lymphoma in early diagnosed, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

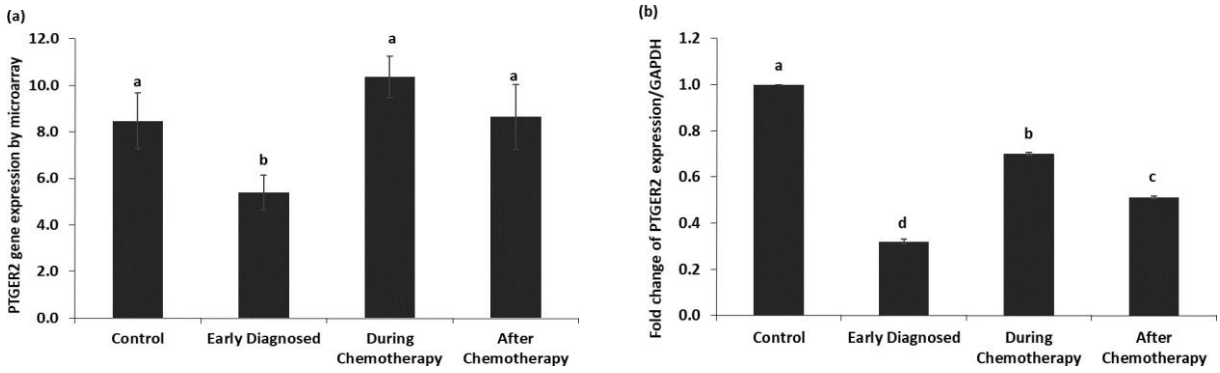

Figure 8. Expression gene PTGER2 (Prostaglandin pathway gene) in lymphoma in early-diagnosed groups, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

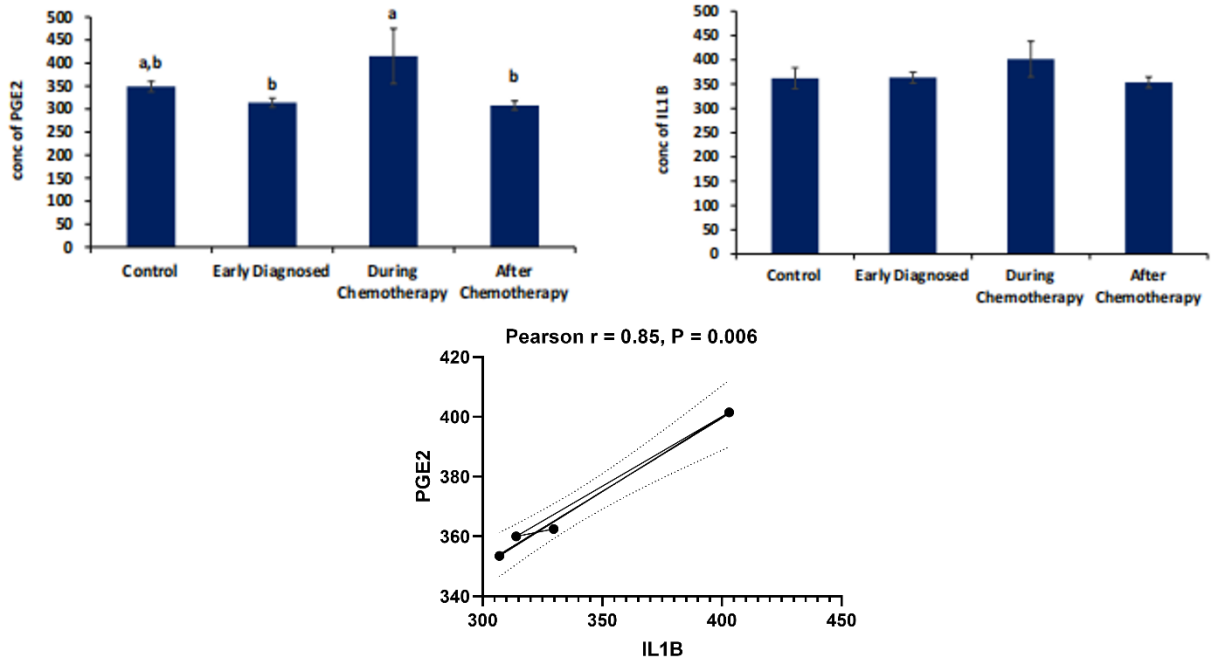

Figure 9. shows concentration level of IL1β (A) and PGE2 (B) in lymphoma patients in early diagnosed, during chemotherapy and after chemotherapy groups. Correlation between IL1β and PGE2 (C). Data was compared Normality of the data was tested with the Kolmogorov-Smirnov test. One–way analysis of variance (ANOVA): Statistically significant at $p < 0.05$. Means that do not share a letter are significantly different.

Furthermore, bermekimab treatment and lower levels of circulating IL-1Ra response in patients with advanced colorectal cancer (Baker, Houston et al. 2019). As regards IL-1α, the analysis shows that the upregulation of the gene expression after induction of chemotherapy. Caspases play a critical role in regulation of apoptosis, cell differentiation, inflammation, and innate immunity, and several are mutated or have altered expression in NHL. It was reported that CASP1 were significantly associated with all major NHL subtypes (Lan, Morton et al. 2009). In our study, we found the gene expression of CASP1 is downregulated in early diagnosed NHL patients compared to healthy control and it upregulated after chemotherapy induction compared to healthy control. Accordingly, upregulation of CASP1 after induction of chemotherapy could illustrate the previous studies.

There are several reports that CCL2 is highly expressed in a variety of cancers and has been linked to a poor prognosis. Inhibiting or blocking the CCL2/CCR2 signaling axis has thus become a focus of cancer therapy (Dasoveanu, Park et al. 2020). On the other hand, a previous study showed that CCL2 was found in high concentrations within the follicular lymphoma (FL)-cell niche. It is also upregulated in mesenchymal stromal cells obtained from healthy age-matched donors mesenchymal stem cells (HD-MSCs) after co-culture with malignant B cells, and it is overexpressed in FL-MSCs. CCL2 expression was also higher in NHL than in reactive lymph nodes, and patients had a shorter survival time (Guilloton et al., 2012). Interestingly, we found the gene expression of CCL2 was downregulated in early-diagnosed NHL patients compared to the healthy control and was upregulated during the chemotherapy group as compared to early-diagnosed group. The role of prostaglandins in lymphoid carcinogenesis is also unknown; however, there is evidence that PGE2 differentially regulates the growth of murine B cell lymphoma. In addition, high levels of PGE have been discovered in lymphoma patients (Inoue et al., 2003). Furthermore, Prostaglandin E2 (PGE2) modulation is important in cancer progression. Prostaglandin is derived from PTGS2 (COX-2) that has been linked to oncogenesis. NSAIDs inhibited PGE2-mediated processes crucial in tumor progression, including tumor cell proliferation, invasion, angiogenesis, and immunosuppression (Wang and Dubois 2006).

It was reported that the expression of COX-2 (PTGS2) and its activity contribute to the pathogenesis of B cell lymphomas, pointing to a possible role for COX-2 inhibition in their treatment (Gandhi et al., 2017). This could illustrate the downregulation of gene expression of PTGS-2 in early-diagnosed NHL patients compared to healthy control. As regards celecoxib, it affects proliferation and sensitizes NHL B-cell lines to apoptosis through COX-2-independent effects by slowing down the cell cycle and decreasing the expression of survival proteins (Gallouet et al., 2014).

In our study, we found that the gene expression of PTGS-2 was regulated during and after the induction of chemotherapy as compared to early diagnosed groups. Furthermore, in non-small cell lung cancer (NSCLC) cell lines, PTGER000 nvc8 34 2 (EP2) expression was downregulated. NSCLCs and adenocarcinomas with PTGER2 methylation had a significantly better prognosis than those without. PTGER2 methylation was more common in tumors with epidermal growth factor receptor (EGFR) mutations than in tumors without EGFR mutation (Liang et al., 2022). Edwards et al., (2012) reported that the precise biologic mechanism of PTGER2 action in tumor cells is still unknown.

PTGER2 has no observed or modified effect in colorectal cancer, but it was downregulated in syntenin-1-knockdown, which is an independent prognostic indicator in CRC (Iwamoto et al., 2020). Additionally, colorectal cancer was found to have EP2 expression in infiltrating neutrophils and tumor-associated fibroblasts. EP2 antagonists suppressed tumorigenesis by amplifying inflammation and shaping the tumor microenvironment, which promotes colon tumorigenesis (Ma et al., 2015). This could illustrate the downregulation of PTGER-2 in early-diagnosed NHL patients compared to Endothelin-1 (ET-1) stimulates COX-1 and COX-2 expression via ETAR, and both enzymes contribute to the production of PGE2 and VEGF (Spinella, Rosano et al. 2004).

Moreover, it was reported that the decrease in ET-1 concentration may be caused by anthracyclines, Anthracyclines have a direct cytotoxic effect, and the decreasing level of ET-1 may play a role in the reduction of the ejection fraction. Zsary, Szucs et al. (2004) illustrated that the downregulation of gene expression of EDN-1 in early diagnosed NHL patients compared to healthy control and upregulated during induction of chemotherapy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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