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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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ABSTRACT

Background: Most lymphoma patients, including non-Hodgkin's lymphoma (NHL), show dysfunction in immunity especially after chemotherapy resulting in a lack of responses to treatment and tumor relapse. 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 the gene profiling of IL-1 and PG pathways in the peripheral blood of NHL patients. **Subjects and Methods:** 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 Center, Tanta. Total RNA (mRNA) was extracted, 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 IL-1 and PG pathways. **Results**: Microarray analysis showed that gene expression of IL-1 pathways, including NFKB1, PELI-1 and CCL2 were 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 the certain genes of IL-1 and Prostaglandin pathways. These inflammatory mediators maybe represented as biomarker in diagnoses and prognosis.

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

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INTRODUCTION

Malignant lymphoma is a proliferative process of the lymphopoietic portion of the reticuloendothelial system, which involves cells of either the lymphocytic or histiocytic series in varying degrees of differentiation that occurs in an effectively homogeneous population of a single cell type (Perez-Ruiz et al., 2013). Non-Hodgkin Lymphomas (NHL) is a heterogeneous group of lymph proliferative malignancies that are much less predictable than Hodgkin's lymphomas and 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 seen involving 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).

Interleukin-1β (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 have the ability to 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 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 of may be due to high expression of inflammatory mediators, 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 has the ability to induce prostaglandin biosynthesis in several cell types associated with inflammation and cancer. We hypothesized that alteration of PG and IL-1 signaling pathways even in the presence of chemotherapy that can be used as diagnostic 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 included of the same ages between 40 and 70 years old of different sex. The patients were grouped in to 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 the blood samples

Five ml of whole venous peripheral blood were 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 centrifugated 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 Clinilab, (EGYPT). The method combines phenol/guanidine-based lysis and silicon membrane-based purification of total RNA.

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

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. 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".

The 5.8s rRNA control probe is the complement to 5.8S rRNA in the samples. Human 5.8S rRNA in the sample (if any), will be labeled with Flash 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 down-regulated 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 evaluated by nanodrop and then, cDNA synthesis performed. Samples with purity ranging from (1.8 to 2.0) were included in the cDNA synthesis protocol. For detection of gene expression, 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) Table 1 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 was considered statistically significant.

RESULTS

NFKB-1 was 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), it was found a decrease in the expression in lymphoma patients by 33.91- fold regardless of the treatment as compared to the healthy control. Its expression showed increases during chemotherapy and after chemotherapy by 10.8, 3.53-folds, respectively as compared to early diagnosis patients, (P-Value <0.001).

Validation of the gene expression using qRT-PCR showed similar trend of 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 expression during chemotherapy group and after chemotherapy were upregulated by 2, 1.4-folds, respectively, as compared to the early diagnosed group, (P-Value <0.001) (Figure 1B).

PELI1 was 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), it was found a decrease in the expression in lymphoma patients by 316.42 -fold regardless the treatment as compared to the healthy control. Its expression showed increases in during chemotherapy and after chemotherapy groups by 76.15, 1.4-folds, respectively, as compared to early -diagnosed patients (P-Value <0.001).

Validation of the gene expression using qRT-PCR showed similar trend 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-as compared to the healthy control. However, the gene expression during 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 was upregulated after chemotherapy in NHL patients

As regards the gene expression of CASP1 (one of IL-1 pathway genes) by gene array analysis (Figure 3A), it was found 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-folds, respectively, as compared to early -diagnosed patients, (P-Value <0.001).

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

IL-1α was upregulated after chemotherapy in NHL patients

As regards gene expression of IL-1α (one of IL-1 pathway genes) by gene array analysis (Figure 4A), It was found a 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 slightly decreases during chemotherapy by 1.21-fold and showed slightly increases after chemotherapy group by -1.4-fold as compared to early -diagnosed patients, (P-Value <0.001).

Validation of the gene expression using qRT-PCR, 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 were upregulated by 4.25, 30.7-folds, respectively, as compared to the early-diagnosed group, (P-Value <0.001) (Figure 4B).

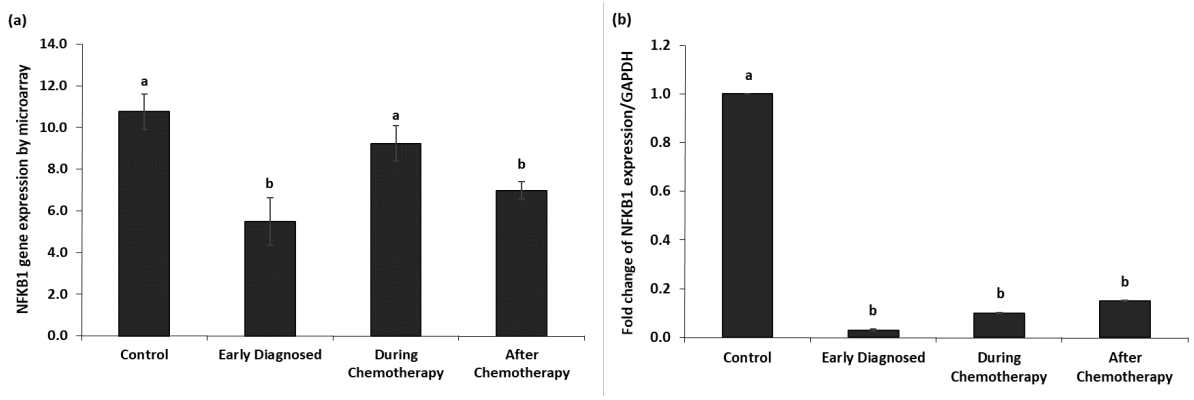

Figure 1. Expression gene of NFKB-1 (IL-1 pathway gene) in lymphoma in early-diagnosed group, during chemotherapy and after chemotherapy groups: A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

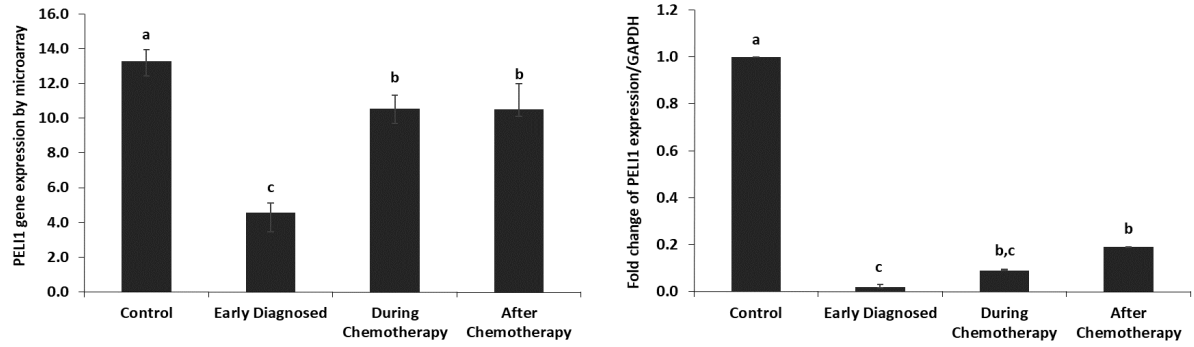

Figure 2. Shows Expression gene PELI1 (IL-1 pathway gene) in lymphoma in early diagnosed group, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

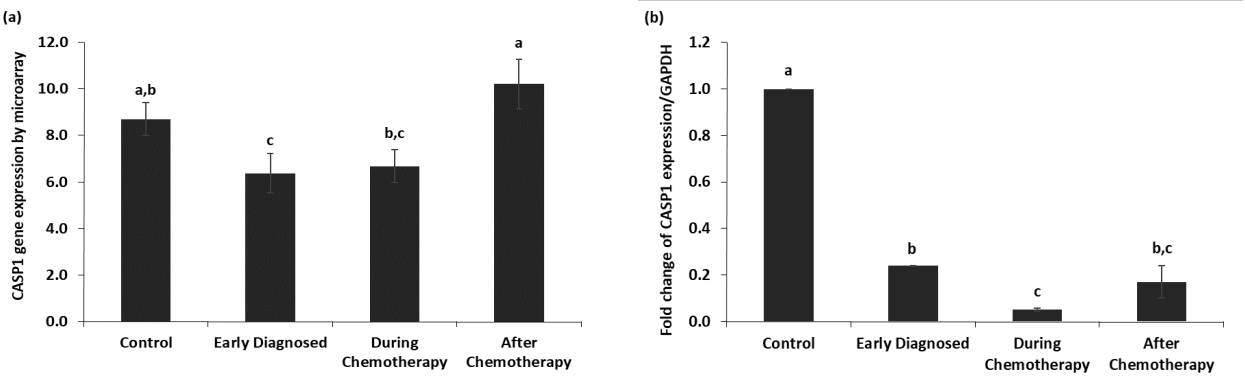

Figure 3. Shows expression gene CASP1 (IL-1 pathway gene) in lymphoma in early diagnosed group, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

CCL2 was upregulated during Chemotherapy in NHL patients

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

Validation of the gene expression using qRT-PCR showed that the fold change of CCL2 expression in the early-diagnosed group was downregulated by 1-fold as compared to the healthy control. The gene expression during chemotherapy group was upregulated 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 was upregulated during and after chemotherapy in NHL patients

As regards gene expression of EDN1 (one of Prostaglandin pathway genes) by gene array analysis (Figure 6A), it was found a decrease in the expression 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-folds and shows decreases after chemotherapy groups by 1.6-folds 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 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).

PTGS-2 was upregulated during and after chemotherapy in NHL patients

As regards gene expression 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 in during chemotherapy and after chemotherapy groups by 1.3, 1.6-folds, 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 expression 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) (Figure 7B).

PTGER-2 was upregulated during and after chemotherapy in NHL patients

As regards the gene expression of PTGER2 (one of Prostaglandin pathway genes) by gene array analysis (Figure 8A), it was found a decrease in the expression in lymphoma patients by 921.3-fold change regardless of the treatment as compared to the healthy control. Its expression showed increases in during chemotherapy and after chemotherapy groups by 824.3, 650.4-folds, 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).

DISCUSSION

Many studies reported that Lymphoma diagnosis and knowledge has 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.

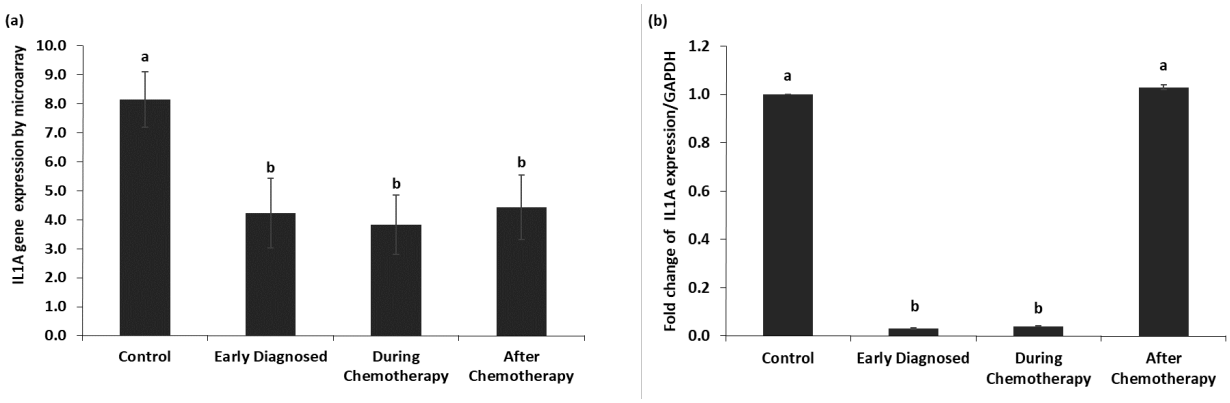

Figure 4. Expression gene of IL-1α (IL-1 pathway gene) in lymphoma in early-diagnosed group, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qrt-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

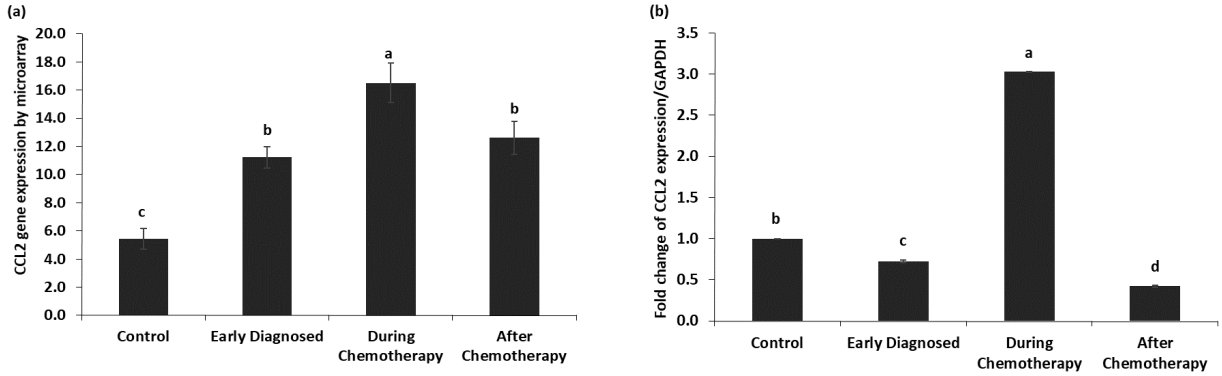

Figure 5. Expression gene of CCL2 (IL-1 pathways gene) in lymphoma in early-diagnosed group, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

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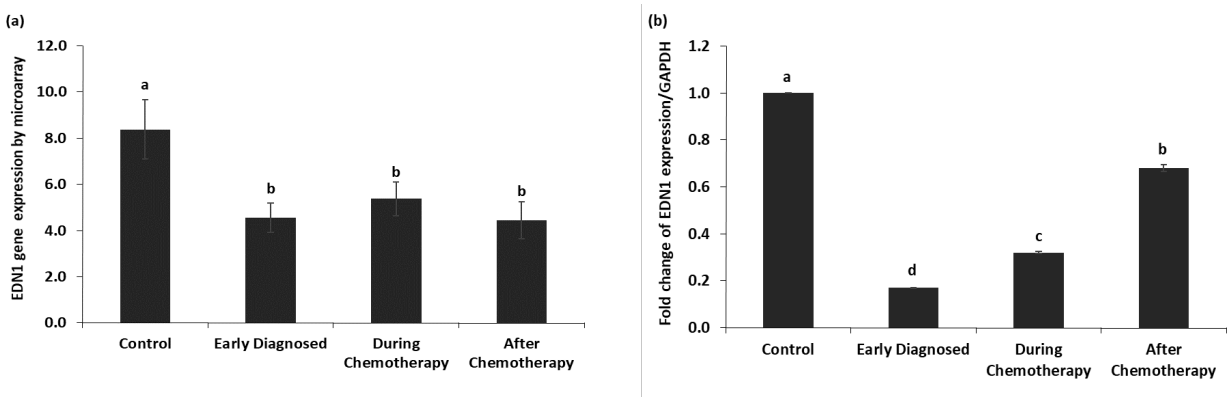

Figure 6. Expression gene of EDN1 (Prostaglandin pathway gene) in lymphoma in early-diagnosed, during chemotherapy and after chemotherapy groups. A) Using Microarray. B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

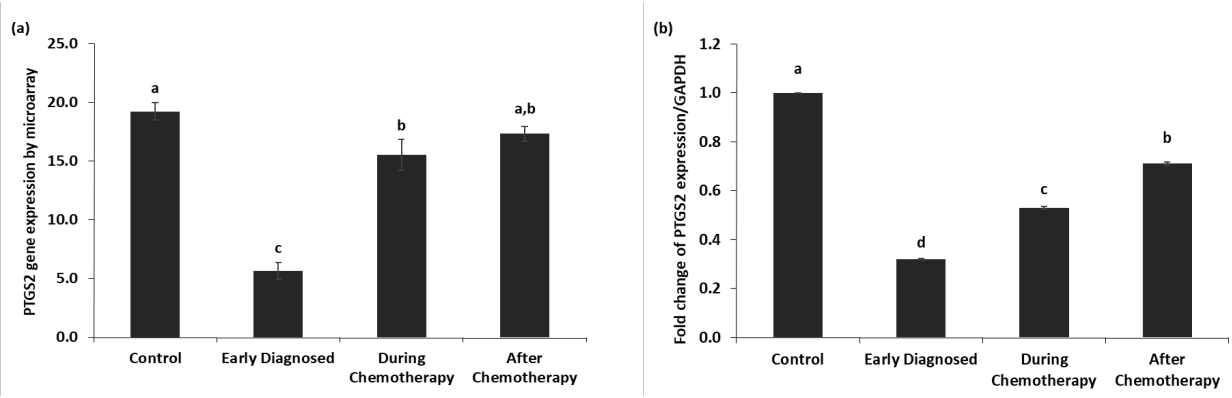

Figure 7. Expression gene of PTGS2 (Prostaglandin pathway gene) in lymphoma in early-diagnosed, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

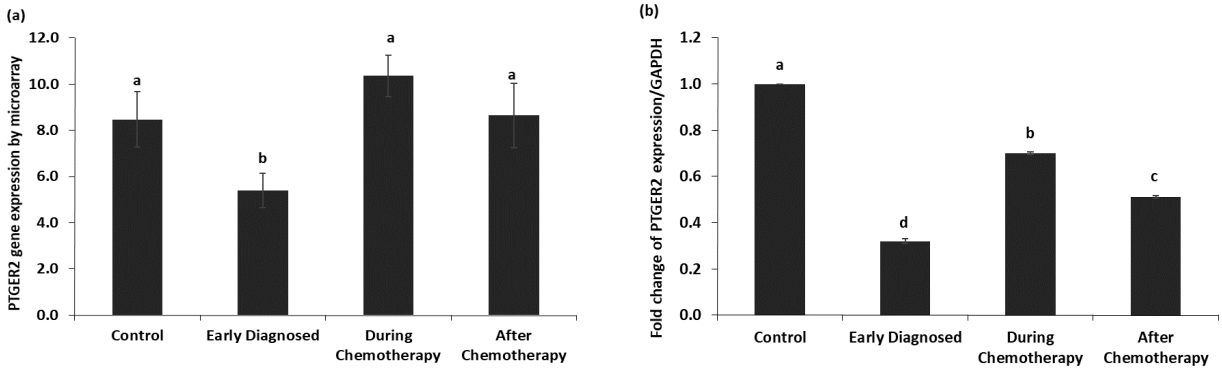

Figure 8. Expression gene PTGER2 (Prostaglandin pathway gene) in lymphoma in early-diagnosed group, during chemotherapy and after chemotherapy groups. A) Using Microarray, B) Using qRT-PCR. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

To characterize this tumor more comprehensively, and to identify new diagnostic and prognostic markers (Kos et al., 2021). Few studies characterized expression profile gene of inflammatory mediators especially prostaglandin including its different types and signaling pathway of critical cytokine such as IL-1 as particularly in cancer patients. In this study, we determined the alteration of expression gene 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 tumor (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 up regulated 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 down regulation 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 group.

There are several reports that CCL2 was 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 side, 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 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 important in tumor progressions, such as tumor cell proliferation, invasion, angiogenesis, and immunosuppression (Wang and Dubois 2006). It was reported, that the expression of COX-2 (PTGS2) and activity, contributes to the pathogenesis of B cell lymphomas and point 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 upregulated during and after the induction of chemotherapy as compared to early-diagnosed group.

Furthermore, in nonsmall cell lung cancer (NSCLC) cell lines, PTGER2 (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

healthy control and upregulated during and after chemotherapy as compared to the early-diagnosed group.

Endothelin-1 (ET-1) stimulates COX-1 and COX-2 expression via ETAR, and both enzymes contribute to the production of PGE2 and VEGF (Tocci, Blandino et al. 2021). Moreover, it was reported that the decrease in ET-1 concentration might 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 (McGowan et al., 2017). It illustrated the downregulation of gene expression of EDN-1 in early-diagnosed NHL patients as compared to healthy control and upregulated during and after induction of chemotherapy as compared to early-diagnosed group.

CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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