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IMMUNOLOGIC ASSESSMENT OF CHEMOTHERAPUTIC EFFECTS MEASURED AS CHANGES IN GENE EXPRESSION LEVELS OF TLRs, MYD88 , AND NF- κ B1 IN PERIPHERAL BLOOD OF NON-HODGKIN LYMPHOMA PATIENTS

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ABSTRACT : Background: Non-Hodgkin Lymphoma (NHL) is a type of cancer that generally develops in the lymph nodes and lymphatic tissue found in organs such as the stomach, intestines or skin , and in some cases involves bone marrow and blood. Cytokines are a broad and loose category of small secreted [proteins](#) and important in [cell signaling](#). They are produced by a wide range of cells, including immune cells like [macrophages](#), [B lymphocytes](#), [T lymphocytes](#) and [mast cells](#), as well as [endothelial cells](#), [fibroblasts](#), and various [stromal cells](#). Interleukins (ILs) are a group of [cytokines](#) that are expressed and secreted by [white blood cells](#) (leukocytes) as well as some other body cells. They have complex immunomodulatory functions including cell proliferation, maturation, migration and adhesion. These cytokines also play an important role in immune cell differentiation and activation. Aim: This study aimed at evaluating the correlation of the interleukins (IL-6 – IL-8 – TNF –IL-12 A and IL-12B) gene expression levels in peripheral blood with chemotherapeutic impact in NHL patients . The most common chemotherapy used for NHL is [CHOP](#), which is a combination regimen of four drugs (Cyclophosphamide, [Hydroxydaunorubicin](#) also called doxorubicin, [Oncovin](#) also called vincristine, and Prednisolone) . Methods: Twenty adult males and females individuals were recruited and used. for this study from the Center of Excellence for Cancer Research (CECR); Tanta University (Tanta, Egypt) were classified into four equal groups (5 individuals each) as follows: Group I: Healthy control volunteers Group II: NHL patients before chemotherapy; Group III: NHL patients after 4 cycles chemotherapy; and Group IV: NHL patients after 6 cycles chemotherapy . The most common chemotherapy used for NHL is [CHOP](#) which is the acronym for a cancer chemotherapeutic drug combination used in the treatment of NHL . It is composed of four drugs (Cyclophosphamide, [Hydroxydaunorubicin](#) also called doxorubicin, [Oncovin](#) also called vincristine, and Prednisolone). NHL patients before chemotherapy, after 4 cycles and after 6 cycles chemotherapy as well as 4 subjects, including 3 lymphoma patients (one patient before ,one after cycles treatment and one after 6 cycles) and 1 healthy controls were enrolled in this study. WBCs were isolated from patients before , after 4 cycles chemotherapy and after 6 cycles chemotherapy. Gene expression of (IL-6 – IL-8 – TNF –12 A and IL-12B) were analyzed by Gene Array Technology. Results: Overall, we found that , the gene expression of IL-6 , IL-8 and TNF showed significant increases in patients before chemotherapy , patient after 4 cycles and patients after 6 cycles chemotherapy than healthy control, however IL-12 gene expression in NHL patients showed no significance difference as compared to healthy controls. We also did not find significant difference in the gene expression of IL-12 in patients before chemotherapy and after both 4 cycles and 6 cycles chemotherapies. Conclusion: The present findings conclude that NHL lymphoma increased the expression of IL-6 , IL-8 and TNF and that chemotherapy alters the expression level of IL-6 and IL-8 but not TNF. These studies form a foundation to understand the possible roles of any IL-6 ,IL-8, TNF , IL-12A , IL-12B in NHL lymphoma.

KEYWORDS: Cancer, Lymphoma, Non-Hodgkin Lymphoma, NHL, Chemotherapy,IL-6 ,IL-8, TNF , IL-12A , IL-12B

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I. INTRODUCTION

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of malignancies that generally develops in the lymph nodes and lymphatic tissue found in organs such as the stomach, intestines or skin . In some cases, NHL involves bone marrow and blood. The majority of the adults NHLs are of B cell in origin while T-cell NHLs are much less common (Jayakrishnan,et al.2008; Perry, et al 2008). Cytokines are soluble proteins that play a key role in all phases of inflammation and regulating immune responses. They play a vital role in activation and differentiation of immune cells and involved in the pathogenesis of many diseases including cancers (Kantola et al., 2012 ,Vainer et al., 2018). IL-6 appears to be one component of a consistent cancer-associated cytokine network resulting in both a systemic immune stimulation and a microenvironment of cancer-induced immune suppression that ultimately protects the cancer cells Interleukin-6 (IL-6) gene encodes a cytokine that association with cancers (Li et al. 2012). Interleukins (ILs) are a group of cytokines that are expressed and secreted by white blood cells (leukocytes) as well as some other body cells. They have complex immunomodulatory functions including cell proliferation, maturation, migration and adhesion. These cytokines also play an important role in immune cell differentiation and activation (Brocker et al. 2010). Both IL-6 and IL-8 are inflammatory chemokines that have been demonstrated to serve an important role in the tumorigenesis of a variety of malignancies, including ovarian cancer (Fu & Lin, 2018). IL-6 and IL-8 are involved in tumor cell apoptosis and invasion, tumor growth and metastasis (Jayatilaka et al., 2017, Yung et al., 2018). IL-8 is a chemokine that mainly targets neutrophils to migrate to the site of infection. It is not only involved in the inflammatory response, but also may act as an important regulatory factor within the tumor microenvironment (Waugh & Wilson, 2008). IL-12 is produced by antigen-presenting cells, such as dendritic cells and macrophages, and is crucial for the recruitment and effector functions of CD8 + T and NK cells (Zundler & Neurath, 2015). Therefore, IL-12 is a major contributor to effective antitumor immune responses (Tugues et al., 2015).

2. SUBJECTS AND METHODS

Subjects

Twenty adults males and females individuals including 5 healthy volunteers plus 15 NHL patients that were recruited and used for this study from the Center of Excellence for Cancer Research (CECR); Tanta University (Tanta, Egypt).. These individuals were classified into four equal groups (5 individuals each) as follows: **Group I:** healthy control volunteers (2 females and 3 males with mean age of 48.4 ± 6.27 years) ; **Group II:** NHL patients before chemotherapy (3 females and 2 males with mean age of 50.50 ± 9.50 years) ; **Group III:** patients after 4 cycles chemotherapy (one female and 4 males with mean age of 60.67 ± 4.65 years); **Group IV:** patients after 6 cycles chemotherapy (3 females and 2 males with mean age of 48.60 ± 5.46 years).

Chemotherapeutic protocol :

The recruited NHL patients were exposed at an interval of three weeks **cycles** (21 days each) to a dose of **CHOP** which is the acronym for a cancer chemotherapeutic drug combination used in the treatment of NHL (**McKelvey et al. 1976**) . It is composed of:

- Cyclophosphamide: It is an [alkylating agent](#) which damages DNA by binding to it and causing the formation of cross-links. It was given as an intravenous infusion at a dose level of 750 mg/m^2 over 30 min on the first day (D1) of treatment.
- [Hydroxydaunorubicin](#) also called doxorubicin : . It is an intercalating agent which damages DNA by inserting itself between DNA bases. It was given as a single intravenous infusion dose Of 50 mg/m^2 over 5-10 min on (D1) of treatment.
- [Oncovin](#) also called vincristine which prevents cells from duplicating by binding to the protein [tubulin](#) . It was given intravenously as a single dose of 2 mg on D1 of treatment .
- Prednisolone: It is a synthetic corticosteroid used to treat a wide range of inflammatory and autoimmune disorders including rheumatic, respiratory, allergic, endocrine, collagen, hematologic, gastrointestinal, and ophthalmic disorders. It was given orally at a dose level of 60 mg/m^2 for 5 consecutive days (D1–D5) of treatment.

Samples collection and WBCs isolation :

Peripheral blood samples (2 ml) were collected into EDTA containing vials. The anti-coagulated blood was diluted with an equal volume of phosphate buffer saline (PBS) and the diluted blood was slowly layered over the Ficoll - Hypaque solution and then centrifugation was applied for 40 min at $400 \times g$, 22°C . The WBCs cells were suspended in PBS. After centrifugation ($100 \times g$ for 10 min at 20°C), the leukocyte cells were suspended in PBS and stored at -80°C for RNA extraction.

RNA Extraction

Immediately after sampling, RNA samples used for the microarrays experiments and RT – qPCR were purified with the RNeasy Kit (Qiagen, Valencia, CA, USA) according to the manufacturer protocol. RNA quality and concentration were determined by using the Nano Drop spectrophotometer (Nano

Technologies).(Chomczynski & Sacchi, 2006). The RNA 6000 Nano Lab Chip Series II Assay used to assess the RNA Integrity. On a Bio Analyzer Agilent 2100This assay generated an RNA integrity number (RIN) for each sample based on the ratio of 28S:18S ribosomal RNA (Schroeder et al., 2006). The samples with (RIN values >8) were used for microarray analysis in the study.

Microarray analysis

Transcriptome analysis was performed using total RNA from each sample. RNA was reverse transcribed using the WT PLUS Reagent Kit (Affymetrix, Santa Clara, CA) according to the manufacturer protocol. Synthesis of biotinylated complementary RNA was performed using a Gene Chip® IVT Labeling kit (Affymetrix) for in vitro transcription.

After fragmentation, 10 µg of complementary RNA was hybridized at 45°C for 16 h on a Gene- Chip® Human Genome U133 Plus 2.0 Array® (Affymetrix). Gene Chips were washed and stained in a Gene Chip® Fluidics Station 450 (Affymetrix). Fluorescence intensities for chips were examined on a Gene Chip Scanner 3000 7G (Affymetrix). Expression Console software (Affymetrix) was used to normalize data and generate probeset intensity values. and Transcriptome Analysis Console (TAC) software (Affymetrix) was used to statistically analyze data . A Benjamini–Hochberg multiple testing correction adjusted *p*-value to smaller than 0.05, in addition a two fold change were used to select genes differentially expressed (Benjamini & Hochberg,1995).

Statistical analysis

The differences between groups were analyzed by ANOVA . The *p* values ≤ 0.05 were considered statistically significant. For normally distributed quantitative variables, to compare between more than two groups, and (Bonferroni) for pairwise comparisons.

Chip Statistical analysis:

Affymetrix Gene Chip operating software (GCOS) (Affymetrix) used to acquire Raw data to yield CEL files. The Affymetrix Expression Console (1.3 .1)(Affymetrix) used to process and analyze the data and gene level differential analysis workflow of Transcriptome Analysis Console(TAC) 3.0(Affymetrix). The back-ground subtraction, normalization, and log base 2 transformation of gene signals were conducted using the robust multi-array average algorithm (Gautier et al., 2004).One-way ANOVA (*p*-value <0.05) were used to compare between the study groups to determine the differentially expressed genes A Benjamini–Hochberg multiple testing correction adjusted *p*-value to smaller than 0.05, in addition a fold change >2 were used to select genes differentially expressed (Benjamini, 2014).

Transcriptomic analysis of selected gene

The result of Transcriptome of the three genes IL-6, IL-8 and TNF in all studied groups, it was found that IL - 6, IL-8 and TNF genes in lymphoma patient showed significance increase in expression level as compared to healthy controls (fold change 70.30 ,fold change 27746.15 and fold change 16.71) (Table 1,2, 3)(figure1A). But there was no significance change IL-12 A and IL-12 B in patient before chemotherapy when compare with healthy control (Fold change 1.59 and fold change -1.01)(figure 1 A).

Impact of chemotherapy on expression of TLR genes.

In patient after 4 cycles chemotherapy (CHOP), there was significance decrease in expression level of IL-6 gene in patients after 4 cycles and 6 cycles chemotherapy as compared to patient before chemotherapy. when we compared the fold change of patient after 6 cycles chemotherapy vs. before chemotherapy found that chemotherapy decrease the expression of IL-6 (Fold change = -45.07). However, this gene in patient after 4 cycles chemotherapy had no change in their expression than 6 cycles is similar(Table1.)(figure 1 B ,1 C) .

IL-8 expression level in patient after 4 cycles show no significance difference as compared to patient before chemotherapy (Fold change = -1.00). IL-8 expression level in patient after 6 cycles show significance decreased as compared to patient before chemotherapy (Fold change = -3.27). However it still more than healthy control (Table 2).

TNF expression level in patient after 4 cycles show significance decrease as compared to patient before chemotherapy (Fold change = - 6.296). TNF expression level in patient after 6 cycles show significance increase as compared to patient before chemotherapy (Fold change = 3.25) (Table 3).

IL-12A and IL-12B expression level in patient after 4 cycles show no significance change as compared to patient before chemotherapy (Fold change = 1.13 and Fold change 1.23).

IL-12A and IL-12B expression level in patient after 6 cycles show no significance change as compared to patient before chemotherapy (Fold change = -1.04 and Fold change = 1.19) We identified that IL-6 and IL-8 genes is down regulated and TNF gene is up regulated in patients after chemotherapy when compare to patients before chemotherapy as shown in (Figure 2)

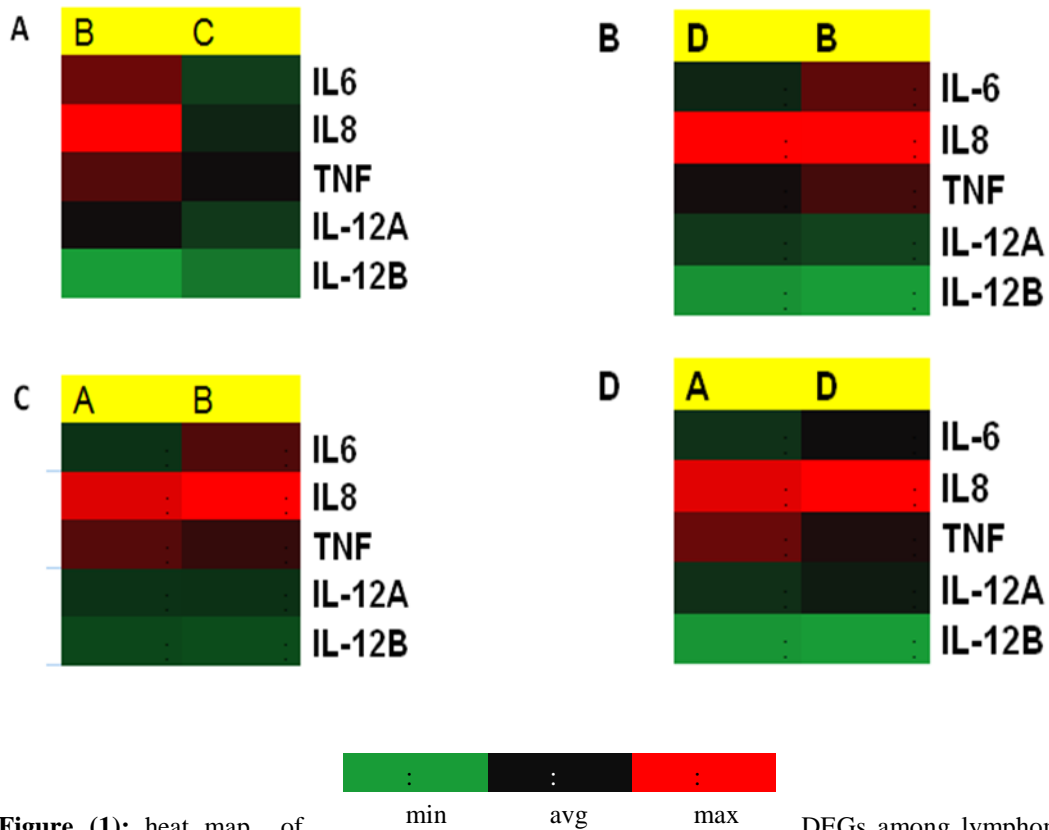


Figure (1): heat map of DEGs among lymphoma patients (before chemotherapy, after 4 cycles chemotherapy and after 6 cycles chemotherapy) and healthy control. DEGs among lymphoma and control groups in (A) healthy control vs. before chemotherapy (B) before chemotherapy vs. after 4 cycles chemotherapy (C) After 6 cycles vs Before chemotherapy (D) after 4 cycles chemotherapy vs. after 6 cycles chemotherapy. DEG, differentially expressed gene

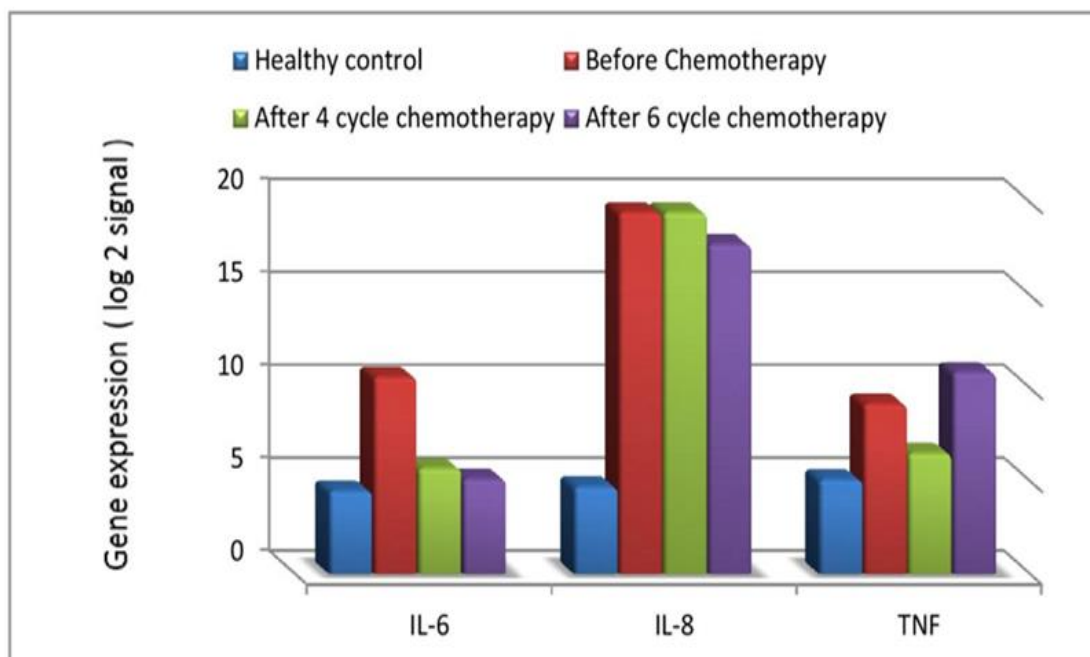


Figure (2) : Chemotherapeutic effect of (CHOP) protocol on selected genes expression (IL-6, IL-8 and TNF) in NHL patients using Microarray

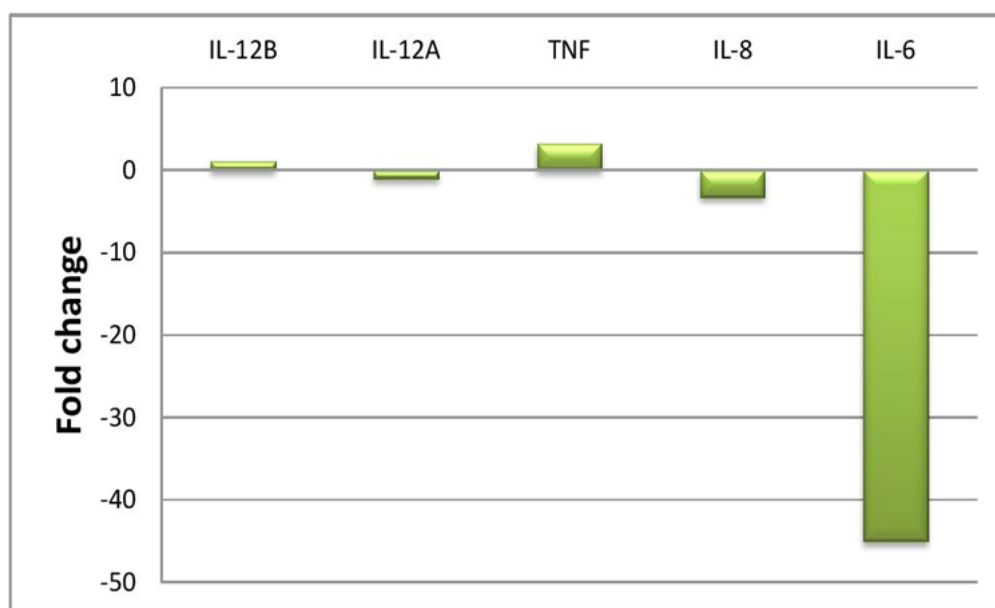


Figure (3) : Differentially expressed genes in patients before chemotherapy vs patient after 6 cycles chemotherapy. up regulated gene (TNF) and Down regulated genes (IL-6 and IL-8)

Table1: Chemotherapeutic effect of Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone(CHOP) protocol every 21 days For 4 cycles (84 days) or 6 cycles(126 days) on IL-6 expression of Non-Hodgkin Lymphoma (NHL) patients.

Groups		IL- 6 (Microarray)	Fold change as compared with healthy control	Fold change compared with patient before chemotherapy	Fold change compared with patients after 4 cycles of chemotherapy
1 st	Healthy control	4.46			
2 nd	Lymphoma Patients before chemotherapy	10.59	70.30		
3 rd	Lymphoma Patients after 4 cycles chemotherapy	5.68	2.33	-30.16	
4 th	Lymphoma Patients after 6 cycles chemotherapy	5.10	1.55	-45.07	-1.49

Table 2: Chemotherapeutic effect of Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone(CHOP) protocol every

21 days For 4 cycles (84 days) or 6 cycles(126 days) on IL-8 of Non-Hodgkin Lymphoma (NHL) patients.

Groups		IL- 8 (Microarray)	Fold change as compared with healthy control	Fold change as compared with patient before chemotherapy	Fold change as compared with patients after 4 cycles of chemotherapy
1 st	Healthy control	4.68			
2 nd	Lymphoma Patient before chemotherapy	19.44	27746.15		
3 rd	Lymphoma Patient after 4 cycl chemotherapy	19.44	27820.29	1.00	
4 th	Lymphoma Patient after 6 cycl chemotherapy	17.72	8472.07	-3.27	-3.28

Table 3: Chemotherapeutic effect of Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone(CHOP) protocol every

21 days For 4 cycles (84 days) or 6 cycles(126 days) on TNF of Non-Hodgkin Lymphoma (NHL) patients.

Groups		TNF (Microarray)	Fold chan as compared wi healthy control	Fold change compared with patien before chemotherapy	Fold change compared wi patients after 4 cycl of chemotherapy
1 st	Healthy control	5.07			
2 nd	Lymphoma Patients befo chemotherapy	9.14	16.71		
3 rd	Lymphoma Patients after 4 cycl chemotherapy	6.48	2.65	-6.29	
4 th	Lymphoma Patients after 6 cycl chemotherapy	10.84	54.44	3.25 +	20.50

Table 4: Chemotherapeutic effect of Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone(CHOP) protocol every

21 days For 4 cycles (84 days) or 6 cycles(126 days) on IL – 12A of Non-Hodgkin Lymphoma (NHL) patients.

Groups		IL – 12A (Microarray)	Fold chan as compared wi healthy control	Fold change compared with patien before chemotherapy	Fold change compared wi patients after 4 cycl of chemotherapy
1 st	Healthy control	4.50			
2 nd	Lymphoma Patient before chemotherapy	5.17	1.59		
3 rd	Lymphoma Patient after 4 cycl chemotherapy	5.36	1.81	1.13	
4 th	Lymphoma Patient after 6 cycl chemotherapy	4.33	1.52	-1.04	-1.18

Table 5: Chemotherapeutic effect of Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone(CHOP) protocol every

21 days For 4 cycles (84 days) or 6 cycles(126 days) on IL – 12B of Non-Hodgkin Lymphoma (NHL) patients.

Groups	IL- 12 B (Microarray)	Fold change as compared with healthy control	Fold change as compared with patient before chemotherapy	Fold change as compared with patients after 4 cycles of chemotherapy
1 st	Healthy control	3.76		
2 nd	Lymphoma Patient before chemotherapy	3.73	-1.01	
3 rd	Lymphoma Patient after 4 cycles chemotherapy	4.03	1.21	1.23
4 th	Lymphoma Patient after 6 cycles chemotherapy	3.98	1.17	-1.03

Discussion

Cytokine may be act as biomarker in disease such as cancer . They are used in diagnosis and classifications between stages of disease. Some examples of cytokines are interleukin- 6 and a tumor necrosis factor-alpha (Abdollahi et al., 2012),(Abdollahi et al., 2012). It is outstanding that some chemotherapies reduced the levels of inflammatory cytokines (Böhm et al., 2016).

IL- 6 is secreted by monocytes and macrophages after stimulation of TLR ligands (Garbers et al., 2018). IL-6 has important role in hematological malignancies such as NHL (Burger, 2013) . A significantly high level of IL-6 observed in our study is in accordance with many reports in other cancers including colorectal cancer (Waldner et al., 2012) and prostate cancer [(Culig & Puhr, 2012)]. This suggests a strong link between IL-6 and cancer. Chemotherapy can induce IL-6 expression in tumor and stromal cells [(C.-T. Wu et al., 2013), 41] via the activation of NF-KB signaling, leading to therapeutic resistance (Wolf et al., 2014).

In this study IL- 6 expression level increase in lymphoma patient (before chemotherapy) than healthy control similar to this studies (Johdi et al., 2020).But IL-6 levels decreased significantly in patients (after 4 cycles and after 6 cycles chemotherapy) than patients (before chemotherapy) similar to this study(Pedersen et al., 2005) and different to this study (Anber et al., 2019) which found insignificant difference compared to pretreatment TNF directly facilitates tumor development by regulating the proliferation and survival of neoplastic cells.

In addition, it indirectly facilitates tumor development via endothelial cells and inflammatory cells present in the tumor microenvironment (Y. Wu & Zhou, 2010)& Zhou, 2010). TNF- α can be used as a powerful biomarker and prognostic factor in (NHL) (Houldsworth et al., 2008). In patients with non-Hodgkin lymphoma higher plasma TNF levels are associated with poorer disease outcomes(Nakayama et al., 2018). In our study there is an increase in expression level of TNF in patient before chemotherapy compare to control similar to this study (Makgoeng et al., 2018) but different to this study (Johdi et al., 2020) .TNF in patient after treatment (after 6 cycle) show significant increase in compare to pre treatment and control different to this study (Anber et al., 2019). The expression of IL-8 in cancer cells is induced by multiple stimuli such as, inflammatory signals (IL-1, TNF- α . (Brat et al., 2005). It is involved in cell proliferation, invasion, and metastasis of many cancers (Burz et al., 2021) (Ha et al., 2017). Furthermore, the overexpression of IL-8 is reported to be correlated with poor prognosis of several solid tumors such as GC(Liu et al., 2018) and breast cancer (Bakouny & Choueiri, 2020). In our study we found increased in expression level of IL-8 in lymphoma patient (before chemotherapy) than healthy control similar to this studies that found increase the expression of IL-8 in gastric cancer (Lin et al., 2022).

After chemo-radiotherapy , the patients with high expression level of IL-8 were significantly higher risk of a local relapse (León et al., 2021) . In our study we found significant decrease in IL-8 expression level after 4 cycle and 6 cycle chemotherapy compare to before chemotherapy but still increased than healthy control Furthermore, IL-12 is capable of activating cytotoxic immune cells (Arau et al., 2017), thus making it a useful substance for the induction of immune response against cancer (de Rham et al., 2007)].

IL-12 is not only an important immunomodulating cytokine but also a strong mediator of cancer development. It exerts proinflammatory functions by activating cytotoxic immune cells [(Balasubramanian et al., 2019)], thus making it an important cytokine in the antitumor response of the immune system (Lu, 2017) .

References

- Abdollahi, A., Shoar, S., Nayyeri, F., & Shariat, M. (2012). Diagnostic value of simultaneous measurement of procalcitonin, interleukin-6 and hs-CRP in prediction of early-onset neonatal sepsis. *Mediterranean Journal of Hematology and Infectious Diseases*, 4(1). <https://doi.org/10.4084/MJHID.2012.028>
- Anber, N. H., El-sebaie, A. H., Shamaa, S. S., Darwish, N. H. E., & Mousa, S. A. (2019). Prognostic value of some inflammatory markers in patients with lymphoma. 0(March), 1–12.
- Arau, S. J., Tirode, F., Coin, F., Pospiech, H., Syva, J. E., Stucki, M., Hu, U., Egly, J., Wood, R. D., Adamo, A., Collis, S. J., Adelman, C. A., Silva, N., Horejsi, Z., Ward, J. D., Martinez-perez, E., Boulton, S. J., Volpe, A. La, Akbari, M., ... Borstel, R. C. J. Von. (2017). 乳鼠心肌提取 HHS Public Access. *Physiology & Behavior*, 176(1), 139–148. <https://doi.org/10.1126/scitranslmed.aaw5680>. Regulatable
- Bakouny, Z., & Choueiri, T. K. (2020). IL-8 and cancer prognosis on immunotherapy. *Nature Medicine*, 26(5), 650–651. <https://doi.org/10.1038/s41591-020-0873-9>
- Balasubramanian, D., Goodlett, B. L., & Mitchell, B. M. (2019). Is IL-12 pro-inflammatory or anti-inflammatory? Depends on the blood pressure. *Cardiovascular Research*, 115(6), 998–999. <https://doi.org/10.1093/cvr/cvz028>
- Benjamini, Y. (2014). <Benjamini&Hochberg1995_FDR.pdf>. June. <https://doi.org/10.2307/2346101>
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57(1), 289–300.
- Böhm, S., Montfort, A., Pearce, O. M. T., Topping, J., Chakravarty, P., Everitt, G. L. A., Clear, A., McDermott, J. R., Ennis, D., Dowe, T., Fitzpatrick, A., Brockbank, E. C., Lawrence, A. C., Jeyarajah, A., Faruqi, A. Z., McNeish, I. A., Singh, N., Lockley, M., & Balkwill, F. R. (2016). Neoadjuvant chemotherapy modulates the immune microenvironment in metastases of tubo-ovarian high-grade serous carcinoma. *Clinical Cancer Research*, 22(12), 3025–3036. <https://doi.org/10.1158/1078-0432.CCR-15-2657>
- Brat, D. J., Bellail, A. C., & Van Meir, E. G. (2005). The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. *Neuro-Oncology*, 7(2), 122–133. <https://doi.org/10.1215/S1152851704001061>
- Brocker C, Thompson D, Matsumoto A, Nebert DW, Vasiliou V (Oct 2010). "[Evolutionary divergence and functions of the human interleukin \(IL\) gene family](#)". *Human Genomics*. 5 (1): 30–55.
- Burger, R. (2013). Impact of Interleukin-6 in hematological malignancies. *Transfusion Medicine and Hemotherapy*, 40(5), 336–343. <https://doi.org/10.1159/000354194>
- Burz, C., Bojan, A., Balacescu, L., Pop, V. V., Silaghi, C., Lupan, I., Aldea, C., Sur, D., Samasca, G., Cainap, C., & Chiorean, B. (2021). Interleukin 8 as predictive factor for response to chemotherapy in colorectal cancer patients. *Acta Clinica Belgica: International Journal of Clinical and Laboratory Medicine*, 76(2), 113–118. <https://doi.org/10.1080/17843286.2019.1680133>
- Chomczynski, P., & Sacchi, N. (2006). The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: Twenty-something years on. *Nature Protocols*, 1(2), 581–585. <https://doi.org/10.1038/nprot.2006.83>
- Culig, Z., & Puhr, M. (2012). Interleukin-6: A multifunctional targetable cytokine in human prostate cancer. *Molecular and Cellular Endocrinology*, 360(1–2), 52–58. <https://doi.org/10.1016/j.mce.2011.05.033>
- de Rham, C., Ferrari-Lacraz, S., Jendly, S., Schneiter, G., Dayer, J. M., & Villard, J. (2007). The proinflammatory cytokines IL-2, IL-15 and IL-21 modulate the repertoire of mature human natural killer cell receptors. *Arthritis Research and Therapy*, 9(6), 1–15. <https://doi.org/10.1186/ar2336>
- Dinarello, C. A. (2000). Proinflammatory cytokines. *Chest*, 118(2), 503–508.
- Fu, S., & Lin, J. (2018). Blocking interleukin-6 and interleukin-8 signaling inhibits cell viability, colony-forming activity, and cell migration in human triple-negative breast cancer and pancreatic cancer cells. *Anticancer Research*, 38(11), 6271–6279. <https://doi.org/10.21873/anticancer.12983>
- Garbers, C., Heink, S., Korn, T., & Rose-John, S. (2018). Interleukin-6: Designing specific therapeutics for a complex cytokine. *Nature Reviews Drug Discovery*, 17(6), 395–412. <https://doi.org/10.1038/nrd.2018.45>
- Gautier, L., Cope, L., Bolstad, B. M., & Irizarry, R. A. (2004). affy — analysis of Affymetrix GeneChip data at the probe level. 20(3), 307–315. <https://doi.org/10.1093/bioinformatics/btg405>
- Ha, H., Debnath, B., & Neamati, N. (2017). Role of the CXCL8-CXCR1/2 axis in cancer and inflammatory diseases. *Theranostics*, 7(6), 1543.
- Houldsworth, J., Petlakh, M., Olshen, A. B., & Chaganti, R. S. K. (2008). Pathway activation in large B-cell non-Hodgkin lymphoma cell lines by doxorubicin reveals prognostic markers of in vivo response. *Leukemia and Lymphoma*, 49(11), 2163–2172. <https://doi.org/10.1080/10428190802381220>
- Husson, H., Lugli, S. M., Ghia, P., Cardoso, A., Roth, A., Brohmi, K., Carideo, E. G., Choi, Y. S., Browning, J., & Freedman, A. S. (2000). Functional effects of TNF and lymphotoxin $\alpha 1\beta 2$ on FDC-like cells. *Cellular Immunology*, 203(2), 134–143.

Jayatilaka, H., Tyle, P., Chen, J. J., Kwak, M., Ju, J., Kim, H. J., Lee, J. S. H., Wu, P.-H., Gilkes, D. M., & Fan, R. (2017). Synergistic IL-6 and IL-8 paracrine signalling pathway infers a strategy to inhibit tumour cell migration. *Nature Communications*, 8(1), 1–12.

Jevtovic-Stoimenov, T., Kocic, G., Pavlovic, D., Mačukanovic-Golubović, L., Marjanovic, G., Djordjevic, V., Tošić, N., & Pavlović, S. (2008). Polymorphisms of tumor-necrosis factor- α - 308 and lymphotoxin- α + 250: Possible modulation of susceptibility to apoptosis in chronic lymphocytic leukemia and non-Hodgkin lymphoma mononuclear cells. *Leukemia & Lymphoma*, 49(11), 2163–2169.

Johdi, N. A., Zulkhairi, H., Said, M., Razif, M., Idris, M., Ismail, N. A., Fariza, W., Jamaludin, W., & Wahid, S. F. A. (2020). *JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY* Cytokine Profiling as A Potential Biomarker in Diffuse Large B-Cell Lymphoma. 8(2), 1–6.

Kantola, T., Klintrup, K., Väyrynen, J. P., Vormanen, J., Bloigu, R., Karhu, T., Herzig, K. H., Näpänkangas, J., Mäkelä, J., Karttunen, T. J., Tuomisto, A., & Mäkinen, M. J. (2012). Stage-dependent alterations of the serum cytokine pattern in colorectal carcinoma. *British Journal of Cancer*, 107(10), 1729–1736. <https://doi.org/10.1038/bjc.2012.456>

León, X., García, J., Farré, N., Majercakova, K., Avilés-Jurado, F.-X., Quer, M., & Camacho, M. (2021). Predictive capacity of IL-8 expression in head and neck squamous carcinoma patients treated with radiotherapy or chemoradiotherapy. *Acta Otorrinolaringologica (English Edition)*, 72(6), 337–343.

Li, J., Mo, H.-Y., Xiong, G., Zhang, L., He, J., Huang, Z.-F., Liu, Z.-W., Chen, Q.-Y., Du, Z.-M., & Zheng, L.-M. (2012). Tumor microenvironment macrophage inhibitory factor directs the accumulation of interleukin-17-producing tumor-infiltrating lymphocytes and predicts favorable survival in nasopharyngeal carcinoma patients. *Journal of Biological Chemistry*, 287(42), 35484–35495.

Lin, L., Li, L., Ma, G., Kang, Y., Wang, X., & He, J. (2022). Overexpression of IL-8 and Wnt2 is associated with prognosis of gastric cancer. *Folia Histochemica et Cytobiologica*, 60(1), 66–73. <https://doi.org/10.5603/FHC.a2022.0002>

Liu, Y., Xu, Y., Wang, Y., Yao, Y., & Yang, J. (2018). Associations between interleukin gene polymorphisms and the risk of gastric cancer: A meta-analysis. *Clinical and Experimental Pharmacology and Physiology*, 45(12), 1236–1244. <https://doi.org/10.1111/1440-1681.13021>

Lu, X. (2017). Impact of IL-12 in Cancer. *Current Cancer Drug Targets*, 17(8), 682–697.

Makgoeng, S. B., Bolanos, R. S., Jeon, C. Y., Weiss, R. E., Arah, O. A., Breen, E. C., Martínez-Maza, O., & Hussain, S. K. (2018). Markers of Immune Activation and Inflammation, and Non-Hodgkin Lymphoma: A Meta-Analysis of Prospective Studies. *JNCI Cancer Spectrum*, 2(4), 1–13. <https://doi.org/10.1093/jncics/pky082>

Marchi, E., & O'Connor, O. A. (2020). The rapidly changing landscape in mature T-cell lymphoma (MTCL) biology and management. *CA: A Cancer Journal for Clinicians*, 70(1), 47–70. <https://doi.org/10.3322/caac.21589>

Nakayama, S., Matsuda, M., Adachi, T., Sueda, S., Ueda, K., Kawahara, K., Ohashi, Y., Awaji, S., Hashimoto, S., & Matsumura, I. (2018). Tumor necrosis factor- and interleukin-6-producing high-grade B-cell lymphoma, not otherwise specified in the pleura. *Leukemia Research Reports*, 10(June), 1–3. <https://doi.org/10.1016/j.lrr.2018.06.001>

Pedersen, L. M., Klausen, T. W., Davidsen, U. H., & Johnsen, H. E. (2005). Early changes in serum IL-6 and VEGF levels predict clinical outcome following first-line therapy in aggressive non-Hodgkin's lymphoma. *Annals of Hematology*, 84(8), 510–516. <https://doi.org/10.1007/s00277-005-1020-x>

Rothman, N., Skibola, C. F., Wang, S. S., Morgan, G., Lan, Q., Smith, M. T., Spinelli, J. J., Willett, E., De Sanjose, S., & Cocco, P. (2006). Genetic variation in TNF and IL10 and risk of non-Hodgkin lymphoma: a report from the InterLymph Consortium. *The Lancet Oncology*, 7(1), 27–38.

Schroeder, A., Mueller, O., Stocker, S., Salowsky, R., Leiber, M., Gassmann, M., Lightfoot, S., Menzel, W., Granzow, M., & Ragg, T. (2006). The RIN: An RNA integrity number for assigning integrity values to RNA measurements. *BMC Molecular Biology*, 7, 1–14. <https://doi.org/10.1186/1471-2199-7-3>

Tugues, S., Burkhard, S. H., Ohs, I., Vrohling, M., Nussbaum, K., Vom Berg, J., Kulig, P., & Becher, B. (2015). New insights into IL-12-mediated tumor suppression. *Cell Death and Differentiation*, 22(2), 237–246. <https://doi.org/10.1038/cdd.2014.134>

Vainer, N., Dehlendorff, C., & Johansen, J. S. (2018). Systematic literature review of IL-6 as a biomarker or treatment target in patients with gastric, bile duct, pancreatic and colorectal cancer. *Oncotarget*, 9(51), 29820–29841. <https://doi.org/10.18632/oncotarget.25661>

Waldner, M. J., Foersch, S., & Neurath, M. F. (2012). Interleukin-6 - A key regulator of colorectal cancer development. *International Journal of Biological Sciences*, 8(9), 1248–1253. <https://doi.org/10.7150/ijbs.4614>

Wang, X., & Liu, Z. (2018). Systematic meta-analysis of genetic variants associated with osteosarcoma susceptibility. *Medicine*, 97(38).

Waugh, D. J. J., & Wilson, C. (2008). The interleukin-8 pathway in cancer. *Clinical Cancer Research*, 14(21), 6735–6741. <https://doi.org/10.1158/1078-0432.CCR-07-4843>

Wen, J., Zhao, Z., Huang, L., Wang, L., Miao, Y., & Wu, J. (2020). IL-8 promotes cell migration through regulating EMT by activating the Wnt/ β -catenin pathway in ovarian cancer. *Journal of Cellular and Molecular Medicine*, 24(2), 1588–1598. <https://doi.org/10.1111/jcmm.14848>

Wolf, J., Rose-John, S., & Garbers, C. (2014). Interleukin-6 and its receptors: a highly regulated and dynamic system. *Cytokine*, 70(1), 11–20.

Wu, C.-T., Chen, M.-F., Chen, W.-C., & Hsieh, C.-C. (2013). The role of IL-6 in the radiation response of prostate cancer. *Radiation Oncology*, 8(1), 1–11.

Wu, Y., & Zhou, B. P. (2010). TNF- α /NF κ -B/Snail pathway in cancer cell migration and invasion. *British Journal of Cancer*, 102(4), 639–644. <https://doi.org/10.1038/sj.bjc.6605530>

Ying, Z., He, T., Wang, X., Zheng, W., Lin, N., Tu, M., Xie, Y., Ping, L., Zhang, C., Liu, W., Deng, L., Qi, F., Ding, Y., Lu, X. an, Song, Y., & Zhu, J. (2019). Parallel Comparison of 4-1BB or CD28 Co-stimulated CD19-Targeted CAR-T Cells for B Cell Non-Hodgkin's Lymphoma. *Molecular Therapy - Oncolytics*, 15(52), 60–68. <https://doi.org/10.1016/j.omto.2019.08.002>

Yung, M. M. H., Tang, H. W. M., Cai, P. C. H., Leung, T. H. Y., Ngu, S. F., Chan, K. K. L., Xu, D., Yang, H., Ngan, H. Y. S., & Chan, D. W. (2018). GRO- α and IL-8 enhance ovarian cancer metastatic potential via the CXCR2-mediated TAK1/NF κ B signaling cascade. *Theranostics*, 8(5), 1270–1285. <https://doi.org/10.7150/thno.22536>

Zundler, S., & Neurath, M. F. (2015). Interleukin-12: Functional activities and implications for disease. *Cytokine & Growth Factor Reviews*, 26(5), 559–568.