Online ISSN: 2682-2628
Print ISSN: 2682-261X

IJCBR

International Journal of Cancer and Biomedical Research

https://jcbr.journals.ekb.eg

Alteration in the gene expression profiling of mitochondrial Electron Transport Chain genes in lymphoma patients

Sohaila M. Khalil, Zeinab I. Attia, Essam Elshiekh, Sameh S. Ali, Mohamed L. Salem

EKB Egyptian Knowledge Bank بنك المعرفة المصري

PUBLISHED BY
EACR EGYPTIAN ASSOCIAN FOR CANCER RESEARCH
Since 2014

International Journal of Cancer & Biomedical Research
(IJCBR) https://jcbr.journals.ekb.eg

IJCBR is an Int. journal published by the Egyptian Society of Cancer Research (EACR, established in 2014, http://eacr.tanta.edu.eg) and sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

IJCBR has been approved by the Supreme Council of Universities, Egypt with score 7 (http://egjournal.scu.eg). The journl is cited by google scholar and registered by Publons (https://publons.com). The journal has recently been evaluated in 2020 by Nature Springer with a good standing.

Scope of IJCBR

- Drug discovery from natural and synthetic resources
- BioMedical applications of nanotehnology
- Sem cell biology and its application
- Basic and applied biotechnology
- Inflammation and autoimmune diseases
- In slico models and bioinformatics
- In vitro and In vivo preclinical animal models
- Cellular and molecular cancer biology
- Cancer Immunology and Immunotherapy
- New methods for prediction, early detection, diagnosis prognosis and treatment of dieases.
- Immunology in health and dieases
- Anti-microbial defense mechanisms
- Cellular and molecular physhiology and pahthology of diseases

IJCBR Editor,
Prof. Mohamed Labib Salem, PhD
Professor of Immunology
Faculty of Science, Tanta Universiy, Egypt

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

Alteration in the gene expression profiling of mitochondrial Electron Transport Chain genes in lymphoma patients

Sohaila M. Khalil[1], Zeinab I. Attia[1], Essam Elshiekh[2], Sameh S. Ali[3], Mohamed L. Salem[1,4]

[1]Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt
[2]Department of Surgical Oncology, Tanta Cancer Center, Tanta, Egypt
[3]Basic Research Department, Children's Cancer Hospital Egypt 57357, Cairo, Egypt
[4]Center of Excellence In Cancer Research (CECR), Tanta University, Tanta, Egypt

ABSTRACT

Background: Many genes are involved in Non-Hodgkin lymphoma (NHL)-associated translocations regulate the cell cycle, apoptosis, and lymphocyte development. NHL commonly occurs along with molecular genetic abnormalities. Certain molecular genetic abnormalities in NHL are related to specific lymphoma subtypes, which are associated with prognosis or potential therapeutic targets. **Aim:** This study aimed to screen for the potential expression of key genes in metabolic pathways in lymphoma patients (NHL) in early diagnosed cases and its clinical relevance. **Materials and Methods:** The relative expression of RNA profiling in 6 early diagnosed NHL patients and 3 healthy control volunteers were determined by using Affymetrix® HTA 2.0 array technology. **Results:** The total number of screened genes was 67528 around 44,699 coding genes in humans and 22,829 non-coding genes for evidence which has shown fundamental significance in normal development, differentiation, regulation of growth and in human diseases such as cancer. Only 6395 genes were differentially expressed between NHL patients and control. From these genes, 451 genes are up-regulated, and 655 genes were down-regulated. Compared to the control group; NHL patients displayed a significant 1.2 fold increase in the expression of mitochondrial biogenesis genes (POLMRT, TFAM) and ATPAse gene (ATP5D). Whereas a significant decrease was observed in the expression of NADH dehydrogenase genes (NDUFS1, NDUFV2, NDUFA1, NSDYFAB1) and mitochondrial biogenesis genes (TFB2M, UCP2, SLC25A) by 1.5 fold. Additionally, cytochrome oxidase genes (COX5A and COX6A1) exhibited a significant 1.3 fold decrease. This expression alteration is associated with cytogenetic abnormalities and may lead to hematological malignancies. **Conclusion:** This study explored the importance of certain mitochondrial related genes which can be used as biomarkers for diagnosis and/or prognosis of the disease.

Keywords: Affymetrix® HTA 2.0 array; genes; metabolic; NHL.

Editor-in-Chief: Prof. M.L. Salem, PhD - Article DOI: 10.21608/JCBR.2020.32420.1044

ARTICLE INFO

Article history

Received: June 15, 2020
Revised: September 11, 2020
Accepted: September 16, 2020

Correspondence to:
Sohaila M. Khalil, MSc
Department Of Zoology,
Faculty Of Science,
Tanta University, Egypt
Phone: +201062306664
Email: sohailagalal37@gmail.com

INTRODUCTION

Non-Hodgkin lymphoma (NHL) is cancer that develops from cells called lymphocytes in the lymphatic system which are a type of white blood cell that helps the body fight infections (Bommannan et al. 2017). For several areas of the body, NHL may grow including the lymph nodes, bone marrow, spleen, thymus, and digestive tract. Otto Warburg showed that tumor ascites had a high rate of glucose consumption and produced lactate, despite sufficient oxygen to completely oxidize glucose (Warburg 1956) ((Liberti and Locasale 2016). This phenomenon has been referred to as the Warburg effect or aerobic glycolysis and is considered a distinctive feature of aggressive tumours. This effect was originally suggested as a result of permanent oxidative metabolism impairment (Warburg 1956). The function of mitochondria in tumor cells was controversial because of many tumor cell lines with high proliferative levels no defects in their oxidative metabolism (Reda et al. 2019). The role of mitochondria in the carcinogenesis process, mainly oxidative phosphorylation, mostly concerns their involvement in the development of free radicals and ATP and the apoptosis

process (Grzybowska-Szatkowska et al. 2014). Few studies have addressed that the lymphoma and mitochondria relationship which leads to aggressiveness and currency. A key intracellular process that generates ATP to power the cell is oxidative phosphorylation (OXPHOS). This is the last step in cellular respiration by which cofactors, such as NADH and FADH2, created the Electron Transport Chain (ETC) to generate H+ gradient that drives ATP synthase to convert ADP to ATP (Wu, Akhtari, and Alachkar 2018). The ETC consists of four complexes and an ATP synthase and is located on the mitochondrial inner membrane. Human mtDNA is present in hundreds to thousands of copies per cell in most tissues and encodes thirteen protein subunits in the inner mitochondrial membrane of four of the five large oxidative phosphorylation (OXPHOS) complexes (Ju et al. 2014). Because of the central function of mitochondria in apoptosis, metabolic control and ROS development in tumor cells, identifying the effects of Electron transmission chain genes in NHL patients may enhance our ability to effectively use these genes in the clinic (Jaramillo et al. 2012). Unfortunately, the expression levels of factors controlling the metabolic pathways in early diagnosed lymphoma patients are not clear. Exploring the contribution of these factors at the gene levels will lead to better design of new strategies not only for diagnosis/prognosis but also for targeted therapy. We aimed to Screen for the expression of key genes in metabolic pathways in lymphoma patients early diagnosed NHL patients as compared to normal lymphocytes from control persons.

MATERIALS AND METHODS

Patients

This study was conducted among a population of NHL Egyptian patients who were recruited before (n = 10; 4 males and 6 females) with a mean age of 60 ± 15.72. Healthy volunteers were also recruited (n = 6; 3 males and 3 females) with a mean age of 35.3 ± 6.3. Patients were recruited from Tanta Cancer Center, Tanta, Egypt, from October 2015 till October 2016. The research study was approved by the Ethical Committee, Faculty of Medicine, Tanta University, Egypt, before the commencement of the study.

Collection of sample

5 ml of whole venous peripheral blood were collected from newly diagnosed patients. Samples were put in ethylenediaminetetraacetic acid (EDTA) tubes. The anticoagulated blood was diluted with an equal volume of PBS and the diluted blood was slowly layered over the Ficoll-Hypaque solution and then centrifugation was applied for 40 min at 400×g, 22°C. The mononuclear cells were suspended in PBS. After centrifugation (100×g for 10min at 20°C), the leukocytes were suspended in PBS and stored at −80°C for RNA extraction.

Diagnosis of Non-Hodgkin lymphoma

Bone marrow aspiration (BMA) is the conclusive tool for the diagnosis of lymphoma. Table 1 shows the mean level of blast cell in patients group was 32.03 %. As regard BMA mean level was 71% in the patient group.

Table 1. Descriptive analysis of early diagnosed cases according to different parameters (Bone Marrow) and tumor marker for diagnosis of NHL.

		Range	Median	Mean±SD
Early diagnosed	Peripheral BLAST (%) day 0	0.0-84.0	37.20±32.03	32.50
	BMA (%) day 0	67.90±20.08	30.0-94.0	71.0
	CD3	CD23	CD20	CD45
Control	Positive	Positive	Positive	Positive
Early diagnosed	Negative	Negative	Positive	Negative

Microarray gene expression profiling

The whole-genome cDNA-mediated annealing, selection and ligation (WG-DASL) was performed using 200 ng RNA. Gene expression data was available for all patients. Samples were analyzed with HGU133+2.0 Affymetrix Gene Chip arrays (Affymetrix). The chips were scanned with an Affymetrix Gene Chip Scanner 3000 and subsequent images were analyzed using Gene Chip R- Operating Software (GCOS) 1.4. Raw feature normalization and quality check were handled using Bioconductor software (affy, affy Q.C Report, GCRMA) (DuBois, Iltis, and Walsh 2019). Data from microarray experiments are representing positive or negative level of expression, relative to some control state, of 1000's of genes for early diagnosed conditions. A short software program is sufficient to translate these data directly into a binary representation suitable as input vectors for the metabolic network.

Statistical analysis

A two-sample *t*-test was used for the analysis of comparing control with early diagnosed patients. A volcano plot was drawn using the negative log of the *p*-value of differentially expressed genes between two groups and log of the fold difference for comparison of gene expressions. Rather than using a fold-change cut-off value to identify differentially expressed genes, we used the fold difference and false-discovery rate of the *p*-value (Q value). The clinical data was collected along with the study and analyzed for each patient; clinical parameters were calculated as the mean ± SD.

The differences between groups were analyzed by the Student's t-test. The p values ≤ 0.05 were considered statistically significant. Linear Correlation coefficient was used for the detection of correlation between two quantitative variables. The differences between groups were analyzed by Mann Whitney test.

RESULTS

Alteration in Gene expression of NHL patients and relative expression in Electron transport chain genes using signal histogram (RMA-DABG)

Signal histogram is a histogram of signal intensity from microarray hybridization. Each value corresponding to each spot is the average of 4 data sets produced by two hybridizations. Robust Multi-array Average (RMA) is background correction, normalization and computation of probe-set level expression. DABG: a detection metric generated by comparing 'Perfect Match' probes to the distribution of background probes. The total number of genes 67528 genes around 44,699 coding genes in humans and 22,829 non-coding genes. From these genes, there were 6359 genes differentially expressed when compared lymphoma patients with healthy control. In addition, 451 genes are up-regulated and 655 genes were down-regulated (Figure **1**A) as representative of heat map of an Affymetrix gene chip and this is the nuclear genome that is associated with cytogenetic abnormalities in lymphoma and its altered expression may lead to NHL lymphoma by causing a deleterious effect on normal immune function in different clusters of genes. We Focused on Electron Transport Chain genes (Figure 2) which include 20 genes which are altered in the chain that differentially changed in the expression.

In this study, we found that mitochondrial genes (TFAM, POLMRT, UCP2 ATP5D, ATP5E, SDHA) are the most consistently upregulated genes in lymphoma patients as compared with healthy control and found that NADH dehydrogenases genes (NDUFS1 and NSDYFAB) and cytochrome oxidase genes (COX5, COX6A1) are most consistently downregulated genes in the same patients (Figure 1B).

Fold change of NADH dehydrogenase and ATPase gene expression in lymphoma

Figure 2 shows NADH dehydrogenases genes, in which the role of each gene (Table 2), the mean of NDUFS1 was represented in early diagnosed group 4.4, but in the control group was 6.6 so it was downregulated by 1.5 fold change (*P*-value = 0.008).

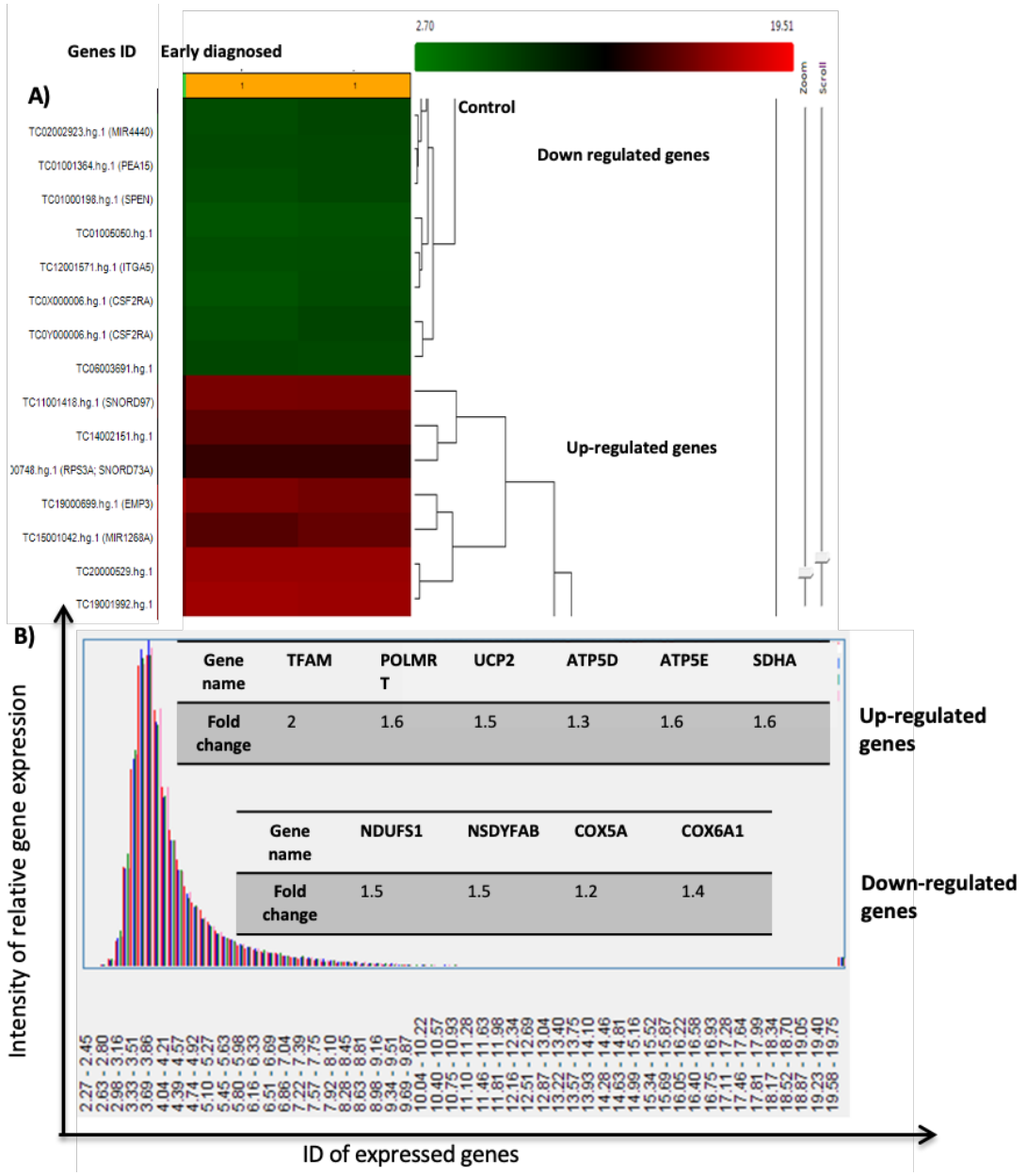

Figure 1A. Representative of Hierarchical clustering of an Affymetrix GeneChip HTA 2.0 array, showing Total RNA microarray gene expression in the peripheral blood of lymphoma patients and healthy control volunteers. B) Representative Signal Histogram (- RMA-DABG –) of the relative expression of Total RNA for Lymphoma patients in different conditions

The mean of NDUFV2 found in the early diagnosed group was 6, and in the control group was 6.6 with significant differences so it was downregulated by 1 fold change (*P*-value = 0.03). The mean of NDUFA1 found in early diagnosed group was 5.2 but in control was 6.9 it was downregulated by 1.3 fold change (*P*-value = 0.04). Moreover, the mean of NSDYFAB1 in the early diagnosed group was 5.6, and in the control group was 8.3 with significant differences so it was downregulated by 1.5 fold change (*P*-value < 0.001). In ATPase, the mean of ATP5D in the early diagnosed group was 7.3 but in the control group was 5.3 with significant differences (*P*-value = 0.014) it was upregulated by 1.3 fold change. However, ATP5E in the patient group early diagnostic group was 7.3 but in the control group was 4.5 so it was upregulated with 1.6 fold change (P-value = 0.019). Transcription genes, the mean of SDHA in the early diagnosed group was 8.9 but in the control group was 5.5 with significant differences (*P*-value = 0.014) it was upregulated by 1.3 fold change.

However, MTIF2 and MITF3 in the early diagnosed group was 6.7 but in the control group was 5.3 it was upregulated with 1.3 fold change so no significant change in the genes (Figure 2).

Fold change of mitochondrial genes expression in lymphoma

The present study showed the mean of cytochrome c oxidase genes (COX6A1) in early diagnosed patients group was 11.6 but in the control group was 8.1 with significant differences (*P*-value = 0.026) so it was downregulated by 1.4 fold change. Moreover, the mean of COX5A in the early diagnosed group was 9.4 but in control was 7.4 so it was downregulated by 1.2 fold change. SURF1 was in early diagnosed group 2.2 but in the control group was 4.1 with significant differences (*P*-value=0.002) so it was downregulated by 1.8 fold change. Interestingly, in mitochondria biogenesis genes, the mean of POLMRT in the early diagnosed group was 6.4 but in control was 3.8 so it was upregulated by 1.6 fold change with significant differences (*P*-value = 0.05). the mean of TFAM in the early diagnosed group was 9.4 but in control was 4 so it was upregulated by 2 fold change with significant differences (*P*-value < 0.001). The mean of TFAM in the early diagnosed group was 9.4 but in control was 4 so it was upregulated by 2 fold change with significant differences (*P*-value<0.001). moreover, the mean of UCP2 in the early diagnosed group was 6.6 but in control was 4 so it was upregulated by 1.5 fold change with significant differences (*P*-value<0.001) (Figure 3).

Correlation between leukocytes and metabolic genes

In Table 2, many cases of early diagnosed of NHL patients, the mean of white blood cells (WBCs) count in control was 7.1 but in the early diagnosed group was 8.4. There is significant change p < 0.001 in SDHA compared to control, In NDUFS1 the significance was P value = 0.09. Moreover, the correlation between COX6A1 and WBCs was significant P-value = 0.05. The percentage of neutrophils in the early diagnosed group was 76.6 but in control was 50, there was a correlation between COX6A and ATP5D with neutrophils P-value < 0.001.

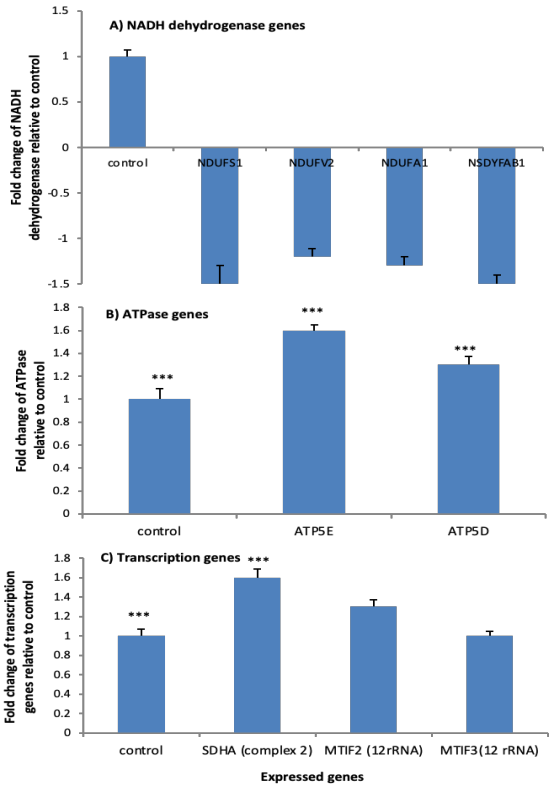

Figure 2. Fold change of NADH dehydrogenase and ATPase genes expression relative to the control: A) Expressed NADH dehydrogenase genes B) Expressed ATPase genes C) Expressed transcription genes. data was expressed in mean ±SE and was compared using t-test, Sig.*: Statistically significant at p < 0.05.

In lymphocytes percentage, it was 10.7 in the early diagnosed group but it was 36.5 in control so SDHA was significant correlation P-value = 0.07. Table 1 shows actual values for each parameter in control and early diagnosed patients.

Correlation between hematological parameters and metabolic genes

The mean of hemoglobin was 14 in the early diagnosed group but 11 in control so the significance of hemoglobin (HB) concentration as shown in supplementary Table 1. There was a correlation in COX6A and ATP5D compared to control p<0.001. There was no change in RBCs in the early diagnosed group as compared with control as shown in supplementary Table 1. The mean of platelet was 204 but in control was 263 as shown in supplementary Table 1 so there was a correlation in NDUFS1 and COX6A1 as shown in Table 3 of platelet count in NHL patients in early diagnosed which was p = 0.001 compared to control.

Table 2. Correlation between genes of metabolic and leukocytes in the early diagnosed group compared to control. The differences between groups were analyzed by Mann Whitney test.

		Genes of metabolic							
		NDUFS1		SDHA		COX6A1		ATP5D	
		r_s	p	r_s	p	r_s	p	r_s	p
WBC (10^3/µl)	Control	-0.029	0.957	-0.886*	0.019*	-0.029	0.957	-0.200	0.704
	Before chemotherapy	-0.866	0.333	0.500	0.667	0.500	0.667	0.50	0.667
NEUT (10^3/µl)	Control	0.771	0.072	-0.714	0.111	-0.086	0.872	0.429	0.397
	Before chemotherapy	-0.866	0.333	0.500	0.667	-1.000*	<0.001*	-1.000*	<0.001*
LYMPH (10^3/µl)	Control	0.486	0.329	-0.257	0.623	0.029	0.957	-0.143	0.787
	Before chemotherapy	-0.866	0.333	0.500	0.667	0.500	0.667	0.50	0.667
MONO (10^3/µl)	Control	0.714	0.111	-0.771	0.072	-0.143	0.787	0.371	0.468
	Before chemotherapy	0.000	1.000	1.000	0.667	-0.500	0.667	-0.500	0.667
EO (10^3/µl)	Control	-0.486	0.329	-0.200	0.704	-0.029	0.957	-0.886	0.019
	Before chemotherapy	0.866	0.333	0.500	0.667	0.500	0.667	0.500	0.667
BASO (10^3/µl)	Control	-0.232	0.658	0.058	0.913	0.174	0.742	-0.348	0.499
	Before chemotherapy	0.866	0.333	0.500	0.667	0.500	0.667	0.500	0.667
NEUT (%)	Control	0.657	0.156	-0.600	0.208	-0.314	0.544	0.543	0.266
	Before chemotherapy	-0.866	0.333	-0.500	0.667	-0.500	0.667	-0.500	0.667
LYMPH (%)	Control	-0.143	0.787	0.657	0.156	0.371	0.468	-0.143	0.787
	Before chemotherapy	0.866	0.333	0.500	0.667	0.500	0.667	0.500	0.667
MONO (%)	Control	0.371	0.468	0.029	0.957	0.200	0.704	0.371	0.468
	Before chemotherapy	0.866	0.333	0.500	0.667	0.500	0.667	0.500	0.667
EO (%)	Control	-0.371	0.468	0.371	0.468	0.314	0.544	-0.543	0.266
	Before chemotherapy	0.500	0.667	-0.866	0.333	0.866	0.333	0.866	0.333
BASO (%)	Control	-0.638	0.173	0.203	0.700	0.319	0.538	-0.551	0.257
	Before chemotherapy	0.866	0.333	0.500	0.667	0.500	0.667	0.500	0.667

WBC: white blood cells , **NEUT**: Neutrophil, **LYMPH**: Lymphocyte, **MONO**: Monocytes, **EO:** Eosinophil, **BASO**: Basophil., r_s: Spearman coefficient, *: Statistically significant at $p < 0.05$

Table 3. Correlation between genes of metabolic and hematological indices in the early diagnosed group compared to control

		Genes of metabolic							
		NDUFS1		SDHA		COX6A1		ATP5D	
		r_s	p	r_s	p	r_s	p	r_s	p
RBC (10^6/µl)	Control	0.771	0.072	-0.543	0.266	0.086	0.872	0.257	0.623
	Before chemotherapy	0.000	1.000	-1.000*	<0.001*	0.500	0.667	0.500	0.667
HGB (g/dl)	Control	0.145	0.784	-0.493	0.321	0.551	0.257	0.377	0.461
	Before chemotherapy	0.000	1.000	-1.000*	<0.001*	0.500	0.667	0.500	0.667
HCT (%)	Control	0.657	0.156	-0.371	0.468	0.257	0.623	0.771	0.072
	Before chemotherapy	0.000	1.000	<0.001*	0.500	0.500	0.667	0.000	1.000
MCV (fL)	Control	-0.435	0.389	-0.464	0.354	0.638	0.173	0.116	0.827
	Before chemotherapy	0.866	0.333	-0.500	0.667	1.000	0.465	1.000	0.0675
MCH (pg)	Control	-0.486	0.329	-0.371	0.468	0.600	0.208	0.086	0.872
	Before chemotherapy	0.000	1.000	-1.000	<0.001*	0.500	0.667	0.500	0.667
MCHC (g/dL)	Control	-0.377	0.461	-0.580	0.228	0.464	0.354	-0.232	0.658
	Before chemotherapy	0.000	1.000	-1.000	0.000	0.500	0.667	0.500	0.667
PLT (10^3/µl)	Control	0.371	0.468	-0.143	0.787	-0.314	0.544	0.543	0.266
	Before chemotherapy	0.000	1.000	1.000	0.034	-0.500	0.667	-0.500	0.667
RDW-SD (fL)	Control	-0.725	0.103	-0.058	0.913	0.522	0.288	-0.174	0.742
	Before chemotherapy	-0.866	0.333	0.500	0.667	-1.000	<0.001*	-1.000	<0.001*
RDW-CV (%)	Control	-0.464	0.354	0.203	0.700	-0.493	0.321	-0.841	0.036
	Before chemotherapy	-0.866	0.333	0.500	0.667	-1.000	<0.001*	-1.000	<0.001*
PDW (fL)	Control	-0.638	0.173	-0.232	0.658	0.174	0.742	-0.696	0.125
	Before chemotherapy	0.866	0.333	-0.500	0.667	1.000	0.667	1.000	0.201
MPV (fL)	Control	-0.600	0.208	-0.314	0.544	0.086	0.872	-0.771	0.072
	Before chemotherapy	-0.866	0.333	-0.500	0.667	-0.500	0.667	-0.500	0.667

RBC: Red blood cells, **HGB**: Hemoglobin, HCT: Hematocrite, **PLT:** Platelets, **MPV:** Mean Platelet Volume, **MCV:** Mean Corpuscular Volume, **MCH:** Mean corpuscular Hemoglobin, **MCHC:** Mean Corpuscular Hemoglobin Concentration, **RDW-CV:** Red Cell Distribution Width, **PDW:** Platelet Distribution Width, r_s: Spearman coefficient, *: Statistically significant at $p < 0.05$.

Table 4. Correlation between genes of metabolic and biochemical parameters in early diagnosed compared to control

		Genes of metabolic							
		NDUFS1		SDHA		COX6A1		ATP5D	
		r_s	p	r_s	p	r_s	p	r_s	p
Urea mg/dl	Control	0.522	0.288	0.232	0.658	-0.058	0.913	-0.058	0.913
	Before chemotherapy	-0.866	0.333	0.500	0.667	-1.000*	<0.001*	-1.000*	<0.001*
GOT/AST (U/L)	Control	0.883	0.020*	-0.412	0.417	-0.177	0.738	0.530	0.280
	Before chemotherapy	-0.866	0.0333	0.500	0.667	0.500	0.0267	0.50	0.0167
GPT/ALT (U/L)	Control	0.794	0.059	0.029	0.956	0.206	0.695	0.912	0.011
	Before chemotherapy	0.866	0..0133	-0.500	0.667	1.000	0.03	1.000	0.02
Creatinine (mg/dL)	Control	0.883	0.020*	0.029	0.956	-0.500	0.312	0.383	0.454
	Before chemotherapy	0.500	0.667	-0.866	0.333	0.866	0.333	0.866	0.333
Bilirubin (mg/dL)	Control	-0.088	0.868	0.441	0.381	-0.265	0.612	0.088	0.868
	Before chemotherapy	0.000	1.000	-1.000	<0.001*	0.500	0.667	0.500	0.667
Uric acid (mg/dL)	Control	0.422	0.188	0.132	0.698	-0.038	0.901	0.038	0.613
	Before chemotherapy	0.866	0.333	0.500	0.667	0.500	0.667	0.500	0.667
LDH (U/L)	Control	0.322	0.088	0.212	0.435	-0.025	1.000	-0.060	0.729
	Before chemotherapy	0.866	0.333	-0.500	0.667	1.000	0.02.	1.000	0.001

GOT: Glutamic oxaloacetic transaminase, **GPT**: Glutamate pyruvic transaminase, **LDH**: Lactate dehydrogenase, r_s: Spearman coefficient, *: Statistically significant at $p < 0.05$

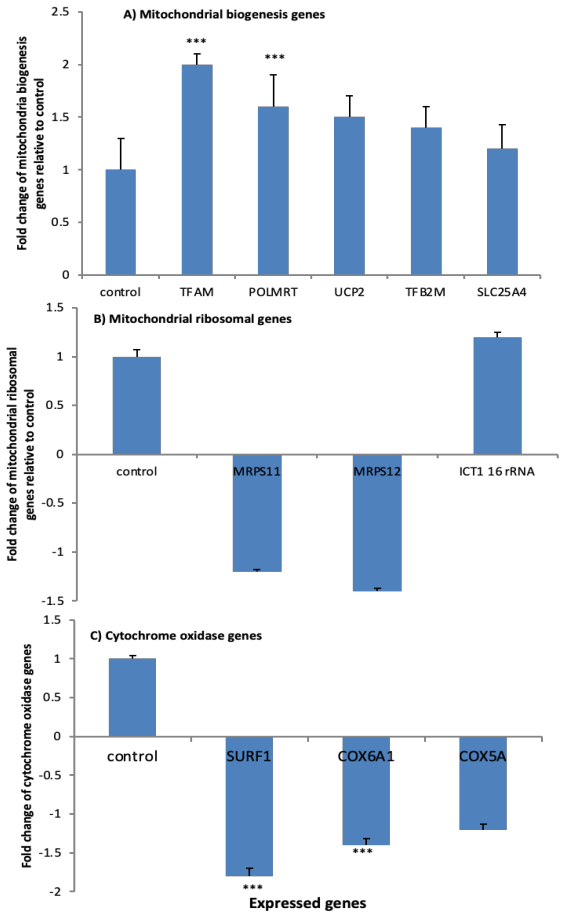

Figure 3. Fold change of mitochondrial gene expression relative to control A) Expressed mitochondrial biogenesis genes B) Expressed mitochondrial ribosomal genes C) Expressed cytochrome C oxidase genes. Expressed data in mean ± SE and was compared using t test (ANOVA), Sig.*: Statistically significant at $p < 0.05$.

Correlation between biochemical parameters and metabolic genes

The mean in urea was 26.7 ± 8.3 in the early diagnosed group and 29.3 ± 4.7 in control in COX6A 1 (Figure 1). The ATP5D was shown a significant correlation in Urea p<0.001* in the early diagnosed group. The mean of GOT and GPT were 39.3 ± 3.2 And 28(10–31) respectively in the early diagnosed group as shown in supplementary Figure 1 but 8.5 ± 1.4 and 7(5-8) In control showing a significant correlation in NDUFS1, COX6A and ATP5D in the early diagnosed group as compared to control group (Table 4). The most significant correlations were in COX6A and ATP5D p<0.001 in the early diagnosed group. The mean of LDH was 390 (325-391) and in control was 180(140-280) (Figure 1), there was a significant correlation in ATP5D and COX6A compared to control p-value=0.001 (Table 4).

DISCUSSION

Microarray technology was widely used in the hope of generating a gene signature that will enable us to predict cancer (Patel and May 2017). Since then, gene expression profiling techniques offer a unique mechanism for interrogating transcriptome-wide levels of gene expression and have proven value in defining gene expression signatures for clinically

important subsets of patients who would otherwise be undetected by conventional prognostication schemes (Nutt et al. 2003). In addition, microarray approaches have been very successful in identifying subsets of tumors that correlate with clinical parameters such as survival, histological grade, and response (Alizadeh et al. 2000). Although many of ETC genes signature have been identified, none of them has been applied to clinical practice due to inconsistencies in the way they were performed in various studies when considering treatment regimens, sample sizes, the patient population studied and definition of complete pathological response (Barshack et al. 2010). Gene expression is controlled on two levels. First, transcription is controlled by limiting the amount of mRNA that is produced from a particular gene. The second level of control is through post-transcriptional events that regulate the translation of mRNA into proteins. Even after a protein is made, post-translational modifications can affect its activity.

The best describe of coding RNA family consists of around 44,699 genes in human and 22, 829 non-coding genes for evidence which has shown fundamental significance in normal development, differentiation, regulation of growth and in human diseases such as cancer. These genes, therefore, influence numerous cancer-related processes such as proliferation, cell-cycle control, apoptosis, differentiation, migration and metabolism (Jansson and Lund 2012). In this study, we determined the relative expression analysis of total RNA profiles in 6 early diagnosed NHL patients. To understand the expression of key genes in metabolic pathways especially Electron Transport chain in early diagnosed patients in lymphoma as compared to normal lymphocytes from healthy control counterparts and its clinical significance, we used a microarray analytical method to combine their expression profiles. The electron transport chain is the ultimate in aerobic respiration and is the only part of the glucose metabolism that requires atmospheric oxygen (Manoj 2018).

In recent studies, deficiency of Mitochondrial complex I demonstrates serve genetic variability and can be caused by a mutation in nuclear-associated genes or genes associated with mitochondria. There are no clear genotype-phenotype associations, and clinical or biochemical inference of underlying basis is difficult, if not impossible (Zhang et al. 2018). Interestingly, we found that Complex 1 such as NDUFS1, NDUV2, NDUFA1 and NSDYFAB are downregulated in gene expression by 1.5 fold in early diagnosed NHL patients compared with control. Succinate dehydrogenase (SDH) is mitochondrial enzyme plays a significant role in both the Krebs cycle and the transport chain of electrons (Casey et al. 2017). In addition, Role of SDHA in normal aerobic respiration, SDH also has tumor-supressive effects. SDHA inactivation results in accumulation of succinate and causes stabilization of the hypoxia-inducible factor (HIF) leading to cell proliferation dysregulation (Burnichon et al. 2017). In our study, we found an elevation in the expression of SDHA by 1.6 fold change in early diagnosed NHL patients as compared with control. Cytochrome c oxidase (COX) is the terminal enzyme of mitochondrial respiratory chain to drive ATP synthesis.

We found that COX5A and COX6A1 are the most consistently downregulated genes in lymphoma patients as compared with healthy control in metabolic pathways. ATP5D and ATP5E encode subunit of mitochondrial ATP synthase.

Mitochondrial ATP synthase catalyzes ATP synthesis using an electrochemical gradient of protons during oxidative phosphorylation across the inner membrane (Black et al. 2020). We found these genes are upregulated. Human mitochondrial transcription factor A (TFAM) has been implicated in promoting tumor growth and invasion. TFAM stimulates transcription of mitochondrial DNA (mtDNA) and regulates the expression of nuclear genes using retrograde mitochondrial signaling. In our study, we found that, compared to control, POLMRT and TFAM are the most significant upregulates genes in the early diagnosed patient group. In conclusion, investigating these genes contribution will contribute to a better design of new approaches, not just for the diagnosis.

Conflict of interest

The authors claim no conflict of interest.

Acknowledgements

This work has been supported by a grant (ID# 5245) funded from Science and Technology Development Fund, Ministry of Scientific Research, Egypt. We would like to express my special thanks to Dr Abdel-Aziz Zidan for his help, support and advice.

References

Alizadeh, A. A., M. B. Eisen, R. E. Davis, C. Ma, I. S. Lossos, A. Rosenwald, J. C. Boldrick, H. Sabet, T. Tran, X. Yu, J. I. Powell, L. Yang, G. E. Marti, T. Moore, J. Hudson, Jr., L. Lu, D. B. Lewis, R. Tibshirani, G. Sherlock, W. C. Chan, T. C. Greiner, D. D. Weisenburger, J. O. Armitage, R. Warnke, R. Levy, W. Wilson, M. R. Grever, J. C. Byrd, D. Botstein, P. O. Brown, and L. M. Staudt. 2000. 'Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling', *Nature*, 403: 503-11.

Barshack, I., G. Lithwick-Yanai, A. Afek, K. Rosenblatt, H. Tabibian-Keissar, M. Zepeniuk, L. Cohen, H. Dan, O. Zion, Y. Strenov, S. Polak-Charcon, and M. Perelman. 2010. 'MicroRNA expression differentiates between primary lung tumors and metastases to the lung', *Pathol Res Pract*, 206: 578-84.

Black, M., P. Arumugam, S. Shukla, A. Pradhan, V. Ustiyan, D. Milewski, V. V. Kalinichenko, and T. V. Kalin. 2020. 'FOXM1 nuclear transcription factor translocates into mitochondria and inhibits oxidative phosphorylation', *Mol Biol Cell*: mbcE19070413.

Bommannan, K., M. U. S. Sachdeva, A. Sekar, R. Kumar, and P. Dey. 2017. 'Primary adrenal T-cell lymphoma in a young adult presented with pseudo-hypopyon: a case report and literature review', *Blood Res*, 52: 227-29.

Burnichon, N., J. M. Mazzella, D. Drui, L. Amar, J. Bertherat, I. Coupier, B. Delemer, I. Guilhem, P. Herman, V. Kerlan, A. Tabarin, N. Wion, K. Lahlou-Laforet, J. Favier, and A. P. Gimenez-Roqueplo. 2017. 'Risk assessment of maternally inherited SDHD paraganglioma and phaeochromocytoma', *J Med Genet*, 54: 125-33.

Casey, R. T., D. B. Ascher, E. Rattenberry, L. Izatt, K. A. Andrews, H. L. Simpson, B. Challis, S. M. Park, V. R. Bulusu, F. Lalloo, D. E. V. Pires, H. West, G. R. Clark, P. S. Smith, J. Whitworth, T. G. Papathomas, P. Taniere, R. Savisaar, L. D. Hurst, E. R. Woodward, and E. R. Maher. 2017. 'SDHA related tumorigenesis: a new case series and literature review for variant interpretation and pathogenicity', *Mol Genet Genomic Med*, 5: 237-50.

DuBois, J. M., A. S. Iltis, and H. A. Walsh. 2019. 'Editors' Note', *Narrat Inq Bioeth*, 9: v-vi.

Grzybowska-Szatkowska, L., B. Slaska, J. Rzymowska, A. Brzozowska, and B. Florianczyk. 2014. 'Novel mitochondrial mutations in the ATP6 and ATP8 genes in patients with breast cancer', *Mol Med Rep*, 10: 1772-8.

Jansson, M. D., and A. H. Lund. 2012. 'MicroRNA and cancer', *Mol Oncol*, 6: 590-610.

Jaramillo, M. C., M. M. Briehl, J. D. Crapo, I. Batinic-Haberle, and M. E. Tome. 2012. 'Manganese porphyrin, MnTE-2-PyP5+, Acts as a pro-oxidant to potentiate glucocorticoid-induced apoptosis in lymphoma cells', *Free Radic Biol Med*, 52: 1272-84.

Ju, Y. S., L. B. Alexandrov, M. Gerstung, I. Martincorena, S. Nik-Zainal, M. Ramakrishna, H. R. Davies, E. Papaemmanuil, G. Gundem, A. Shlien, N. Bolli, S. Behjati, P. S. Tarpey, J. Nangalia, C. E. Massie, A. P. Butler, J. W. Teague, G. S. Vassiliou, A. R. Green, M. Q. Du, A. Unnikrishnan, J. E. Pimanda, B. T. Teh, N. Munshi, M. Greaves, P. Vyas, A. K. El-Naggar, T. Santarius, V. P. Collins, R. Grundy, J. A. Taylor, D. N. Hayes, D. Malkin, Icgc Breast Cancer Group, Icgc Chronic Myeloid Disorders Group, Icgc Prostate Cancer Group, C. S. Foster, A. Y. Warren, H. C. Whitaker, D. Brewer, R. Eeles, C. Cooper, D. Neal, T. Visakorpi, W. B. Isaacs, G. S. Bova, A. M. Flanagan, P. A. Futreal, A. G. Lynch, P. F. Chinnery, U. McDermott, M. R. Stratton, and P. J. Campbell. 2014. 'Origins and functional consequences of somatic mitochondrial DNA mutations in human cancer', *Elife*, 3.

Liberti, M. V., and J. W. Locasale. 2016. 'The Warburg Effect: How Does it Benefit Cancer Cells?', *Trends Biochem Sci*, 41: 211-18.

Manoj, K. M. 2018. 'Aerobic Respiration: Criticism of the Proton-centric Explanation Involving Rotary Adenosine Triphosphate Synthesis, Chemiosmosis Principle, Proton Pumps and Electron Transport Chain', *Biochem Insights*, 11: 1178626418818442.

Nutt, C. L., D. R. Mani, R. A. Betensky, P. Tamayo, J. G. Cairncross, C. Ladd, U. Pohl, C. Hartmann, M. E. McLaughlin, T. T. Batchelor, P. M. Black, A. von Deimling, S. L. Pomeroy, T. R. Golub, and D. N. Louis. 2003. 'Gene expression-based classification of malignant gliomas correlates better with survival than histological classification', *Cancer Res*, 63: 1602-7.

Patel, U. J., and M. May. 2017. 'Lenvatinib in the Treatment of Differentiated Thyroid Cancer

and Advanced Renal Cell Carcinoma', *J Adv Pract Oncol*, 8: 757-64.

Reda, A., A. Refaat, A. A. Abd-Rabou, A. M. Mahmoud, M. Adel, S. Sabet, and S. S. Ali. 2019. 'Role of mitochondria in rescuing glycolytically inhibited subpopulation of triple negative but not hormone-responsive breast cancer cells', *Sci Rep*, 9: 13748.

Warburg, O. 1956. '[Origin of cancer cells]', *Oncologia*, 9: 75-83.

Wu, S., M. Akhtari, and H. Alachkar. 2018. 'Characterization of Mutations in the Mitochondrial Encoded Electron Transport Chain Complexes in Acute Myeloid Leukemia', *Sci Rep*, 8: 13301.

Zhang, S. M., L. Bu, M. R. Laidemitt, L. Lu, M. W. Mutuku, G. M. Mkoji, and E. S. Loker. 2018. 'Complete mitochondrial and rDNA complex sequences of important vector species of Biomphalaria, obligatory hosts of the human-infecting blood fluke, Schistosoma mansoni', *Sci Rep*, 8: 7341.

Egyptian Association for Cancer Research (EACR)
http://eacr.tanta.edu.eg/

EACR is an NGO society that was declared by the Ministry of Social Solidarity (Egypt) No. 1938 in 19/11/2014 based on the initiative of Prof. Mohamed Labib Salem, the current Chairman of EACR. EACR aims primarily to assist researchers, in particular young researchers in the field of cancer research through workshops, seminars and conferences. Its first international annual conference entitled "Anti-Cancer Drug Discovery" was successfully organized in April 2019 (http://acdd.tanta.edu.eg). Additionally, EACR aims to raise the awareness of the society about the importance of scientific research in the field of cancer research in prediction, early diagnosis and treatment of cancer. EACR is also keen to outreach the scientific community with periodicals and news on cancer research including peer-reviewed scientific journals for the publication of cutting-edge research. The official scientific journal of EACR is "International Journal of Cancer and biomedical Research (IJCBR: https://jcbr.journals.ekb.eg) was successfully issued in 2017 and has been sponsored by the Egyptian Knowledge Bank (EKB: www.ekb.eg).

EACR Chairman,
Prof. Mohamed Labib Salem, PhD
Professor of Immunology
Faculty of Science, Tanta Universiy, Egypt

International Journal of Cancer & Biomedical Research
(IJCBR) Online ISSN 2682-2628

For more information, contact

Hamdi Kandil
Tanta University, Egypt
Email: Ijcbr100@gmail.com