ASPI | Afarand Scholarly Publishing Institute; Iran
ISSN: 2008-2630 Iranian Journal of War & Public Health 2022;14(1):75-81 DOI: 10.29252/ijwph.14.1.75

Impact of Gene Expression of TLR4, TLR7, and TLR9 in Children with Acute Lymphocytic Leukemia in Basrah

ARTICLE INFO

Article Type
Original research

Authors
Mustafa R.A.[1] *MSc,*
Jasim H.A.*[1] *PhD,*
Ali Al-Salait S.Kh.[2] *PhD*

How to cite this article
Mustafa R A, Jasim H A, Ali Al-Salait S Kh. Impact of Gene Expression of TLR4, TLR7, and TLR9 in Children with Acute Lymphocytic Leukemia in Basrah. Iranian Journal of War & Public Health. 2022;14(1):75-81.

ABSTRACT

Aims Acute lymphoblastic leukemia (ALL) is the most prevalent malignancy in children, accounting for up to 25% of all malignancies in children under the age of 15. TLRs are associated with the transduction of molecular signals in immune processes such as the production of cytokines, and recognition of specific molecular patterns on the surface of microorganisms, but they are also involved in cancer development. This study was trying to throw light on any possible association of gene expression of TLR4, TLR7, and TLR9 in pediatric patients with ALL.

Materials & Methods A case-control study was conducted on pediatric patients with ALL who have been admitted to Al-Basra Children Teaching Specialty Hospital. Over a period from September 2020 through June 2021, 62 patients (42 newly diagnosed and 20 relapses) were enrolled, in addition to 60 matched normal control, aged 6 months to 16 years. Three ml of blood was collected from all participants in EDTA tubes used for RNA extraction and then molecular analysis. Gene expression of TLR4, TLR7, and TLR9 was done by Real Time-qPCR and the results were reported as ΔCt (mean±SD).

Findings The mean ΔCt of TLR7 (-5.2200±3.29806) reflects the high expression of the gene being the most highly expressed gene ($p<0.001$). The mean ΔCt±SD of TLR7 and TLR9 are high in a newly diagnosed group than relapsed one with no significant differences ($p=0.686$, and 0.400) respectively, while the mean ΔCt of TLR4 is higher significantly ($p<0.05$) in a newly diagnosed group than relapsed one.

Conclusion TLR4, TLR7, and TLR9 gene expression are higher in ALL patients, whether newly diagnosed or relapsed than in the control group. TLRs expression might be part of the immune-evasion mechanism developed by the malignant cells that play an important role in leukemogenicity and disease progression.

Keywords Quantitative Real Time PCR; Toll-Like Receptors; ALL Childhood

CITATION LINKS

[1] Leukemia [2] The 2016 revision to the ... [3] Febrile neutropenia in acute ... [4] New challenges in targeting ... [5] Rates and trends of childhood ... [6] Platelet indices as markers for remission ... [7] Evaluation of VEGF-A in relation ... [8] Quantitative determination of serum ... [9] Prognostic impact of age in children and ... [10] The incidence peaks of the ... [11] Toll-like receptors (TLRs):structure ... [12] Recent progress in the development ... [13] Signaling organelles of the ... [14] Functional toll-like receptors ... [15] Overexpression of TLR3, TLR4, TLR7 ... [16] IL-23/IL-27 Ratio in Peripheral Blood ... [17] Children's experiences of Cancer ... [18] Leukemia study in Sulaymaniyah ... [19] Childhood leukemia rates climb in southern ... [20] Trends in childhood leukemia ... [21] Acute myeloid leukaemia and the ... [22] The biology of toll-like receptor ... [23] Low expression of Toll-like receptors in ... [24] Toll-like receptors (TLRs): An old family of immune ... [25] The secretion of IL-6 by CpG-ODN-treated ... [26] Quantitative expression of Toll-like receptor-2, -4, and -9 in ... [27] Toll-like receptors and ... [28] Gene Polymorphisms and Febrile ... [29] The significance of serum interleukin-10 on ... [30] Endoplasmic reticulum stress induces ... [31] Anthocyanins as inflammatory ... [32] Nucleic acid-sensing toll-like receptors 3, 7 and 8 in ... [33] Triggering of TLR7 and TLR8 expressed by human ... [34] Differential cytokine and Toll-like ... [35] Quantitative expression of toll-like ... [36] Crosstalk between the HIF-1 and ... [37] Roles of toll-like receptors in cancer ... [38] The expression of Toll like ... [39] Distinct TLR-mediated cytokine ... [40] The role of TLR8 signaling ... [41] Expressional correlation of Toll-like Receptor 9 ... [42] Umeda S. Differential expression of ... [43] TLR9 signaling in the tumor ... [44] TLR9 drives the development of ... [45] Heterogeneity of Toll-like ... [46] Toll-like receptor 9 negatively ...

[1]Department of Microbiology, College of Medicine, University of Basrah, Basrah, Iraq
[2]Department of Pathology, Al-Zahraa Medical College, University of Basrah, Basrah, Iraq

***Correspondence**
Address: Department of Microbiology, College of Medicine, Al Bradheia, Al Bradheia st., Basrah, Iraq
Phone: +96 (47) 801169880
Fax: -
hanadi.jasim@uobasrah.edu.iq

Article History
Received: March 7, 2022
Accepted: March 26, 2022
ePublished: May 1, 2022

Introduction

Leukemia is defined as a hematopoietic cell proliferation that invades the bone marrow and spreads to the circulation and other organs [1]. Lymphocytic and myeloid cells are produced by hematopoietic stem cells. B-cells, T-cells, and natural killer cells are lymphoid cells, while erythrocytes, megakaryocytes, neutrophils, eosinophils, basophils, macrophages, and monocytes are myeloid cells [2]. Hematopoietic lineages (Hb, platelets, and neutrophils) are normal, but they have an unfavorable effect on blood cell formation and immunity. As a result, people with acute leukemia are more sensitive to infectious complications [3].

Acute Lymphocytic Leukemia (ALL) is a malignancy of B or T lymphoblasts characterized by uncontrolled proliferation of abnormal, immature lymphocytes and their progenitors which ultimately leads to the replacement of bone marrow elements and other lymphoid organs resulting in a typical disease pattern characteristic of Acute Lymphocytic Leukemia [4]. Acute lymphoblastic leukemia (ALL) is the most prevalent malignancy in children, accounting for up to 25% of all malignancies in children under the age of 15 [5]. A higher risk of ALL is in childhood patients aged 1 to 10 years. It was observed that the majority of ALL pediatric ages range from 2 to 12 with percentages (38% and 36%) respectively [6-8]. Clinical outcome is related to the age of diagnosis, infants and adolescents have a worse prognosis [9]. The better prognosis noted in children aged 1 to 10 years is attributable to the presence of favorable cytogenetic characteristics, e.g. hyperdiploidy or translocation t (12;21) in leukemic blasts [10].

TLRs are integral membrane type I glycoproteins with 3 main domains: an extracellular domain with various numbers of leucine-rich repeat (LRR) motifs, a transmembrane domain, and a cytoplasmic domain (similar to that of the interleukin-1 receptor; IL-1R) known as the Toll/IL-1R (TIR) domain. TLRs have a direct role in the control of inflammatory reactions as well as the activation of innate or adaptive immune responses to eliminate pathogenic microorganisms and cancer debris [11]. TLRs which are found in bacteria, viruses, fungi, and parasites, are components of the immune system and recognize pathogen- and danger-associated molecular patterns [12]. Several of the TLR type (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11) are contained in the plasma membrane, while others are located in endosomes (TLR3, TLR7, TLR8, and TLR9). Two signaling pathways are dependent on MyD88 protein and signaling pathways that are not [13]. TLRs homo or heterodimerize in response to ligand binding, resulting in the recruitment of adaptor proteins-the most prevalent of which is MyD88, which is shared by all TLRs except TLR3 [14]. TLRs are associated with the transduction of molecular signals in immune processes such as induction and regulation of immunity, production of cytokines, and recognition of specific molecular patterns on the surface of microorganisms, but they are also involved in cancer development, as evidenced by previous studies.

Because there are few specific researches on TLR expression in Acute Lymphoblastic leukemia at different phases of progression, this study was tried to shed light on any possible association of gene expression of TLR4, TLR7, and TLR9 in pediatric patients with the pathogenesis of acute lymphocytic leukemia and compared to that in normal matched children.

Materials & Methods

A case-control study was conducted on pediatric patients with Acute Lymphocytic Leukemia who have been admitted to Al-Basra Children Teaching Specialty Hospital. Over a period from September 2020 through June 2021, 62 patients (42 newly diagnosed and 20 relapses) were enrolled, in addition to 60 matched normal control, aged 6 months to 16 years. Three ml of blood was collected from all participants in EDTA (Ethylene Diamine Tetra Acetic acid) tubes used for RNA extraction and then for molecular analysis. TLR4, TLR7, and TLR9 gene expression were done by Real Time q-PCR, qTOWER3 machine from Analytic-Jeana. The procedure included the RNA extraction step by using kits supplied by Wizbio solution (Korea), in addition, to the synthesis of the cDNA step by using the kits supplied by WizScript RT FDmix (Hexamer). Specific oligonucleotides sequence of primers for each TLR4, TLR7, and TLR9 gene and β-actin (housekeeping gene) were used (Table 1). Optimization and solving of the primers depended on the instruction supplied by the source. Then quantification was measured by using Real-Time qPCR. The conditions for amplification of TLRs genes were as in Table 2.

The data of the current study were analyzed by using Statistical Package of Social Science (SPSS) version 26. Analyzed the data of the current study by using Chi-square (X^2) test to compare percentages. Numeric data were described by Mean±SD.

Table 1) Primers of TLRs genes used in the study

TLRs	Forward	Reverse	Length (bp)	
			F	R
TLR4	5'TCTTCAACCAG ACCTCTACATTCC A3'	5'GGAACATCCAGA GTGACATCACAG3'	25	24
TLR7	5'CCGTGACAATT ACCTGGCCTTC3'	5'CAGGGCCTTCAG CTGGTTTC3'	22	20
TLR9	5'AGGATGATGCC AGGATGATGTC3'	5'TCAGGTCCAGGT TCTTGGTTGAG3'	22	23
β-actin	5'GGCACCCAGCA CAATGAAG3'	5'CCGATCCACACG GAGTACTTG	19	21

Table 2) Conditions of Real-Time-q PCR used for amplification of TLRs genes [16]

The stage	Temperature (°C)	Time (Sec.)	Number of cycles
Initial denaturation	95	600	40
Denaturation	95	15	
Annealing	55	30	
Extension	60	60	

Findings

Gene expression of TLRs (TLR4, TLR7, and TLR9) of study groups was analyzed (Table 3) by using Real Time-qPCR, and the results were reported as ΔCt (mean±SD), as shown in Table 3. The mean ΔCt of TLRs reflects the high expression of a gene with the TLR7 being the most highly expressed gene ($p<0.001$). In general, the mean ΔCt of TLRs were stated to be high in ALL patients than in the control group.

TLRs gene expression in newly diagnosed ALL patients compared to relapsed ALL patients was measured (Table 4). The mean ΔCt±SD of TLR7 and TLR9 are high in both groups with no significant differences (P-value 0.686 and 0.400) respectively, while the mean ΔCt±SD of TLR4 is higher in a newly diagnosed group (2.90524±1.02402) than relapsed one (6.49050±1.91184) with significant differences <0.001.

In real-time polymerase chain reaction for Toll-like receptors gene expression (Figure 1) shows the starting time of amplification as it was after 20 cycles/min. Also (Figure 1-E) shows the melting curve analysis of TLRs and the temperature of cycles (50C° for 1 minute). The intercalating dye was SYBR green.

Table 3) The Mean ΔCT*±SD of TLRs gene expression in ALL pediatric patients and control group measured by real time-qPCR (N=122)

Parameter	ALL Patients	Control	p-value
TLR4	4.0618±2.16754	11.3295±1.45013	0.000
TLR7	-5.2200±3.05991	10.0243±1.38014	0.000
TLR9	4.3168±1.17849	10.9862±1.43895	0.000

* lower ΔCt value indicates that the gene expression is high

Table 4) The Mean ΔCT*±SD of TLRs gene expression in both new cases and relapse ALL patients (N=62)

Parameters	New cases	Relapse cases	p-value
TLR4	2.90524±1.02402	6.49050±1.91184	0.000
TLR7	-5.11048±3.29806	-5.45000±2.55071	0.686
TLR9	4.22905±1.00882	4.5010±1.48662	0 .400

*lower ΔCt value indicates that the gene expression is high

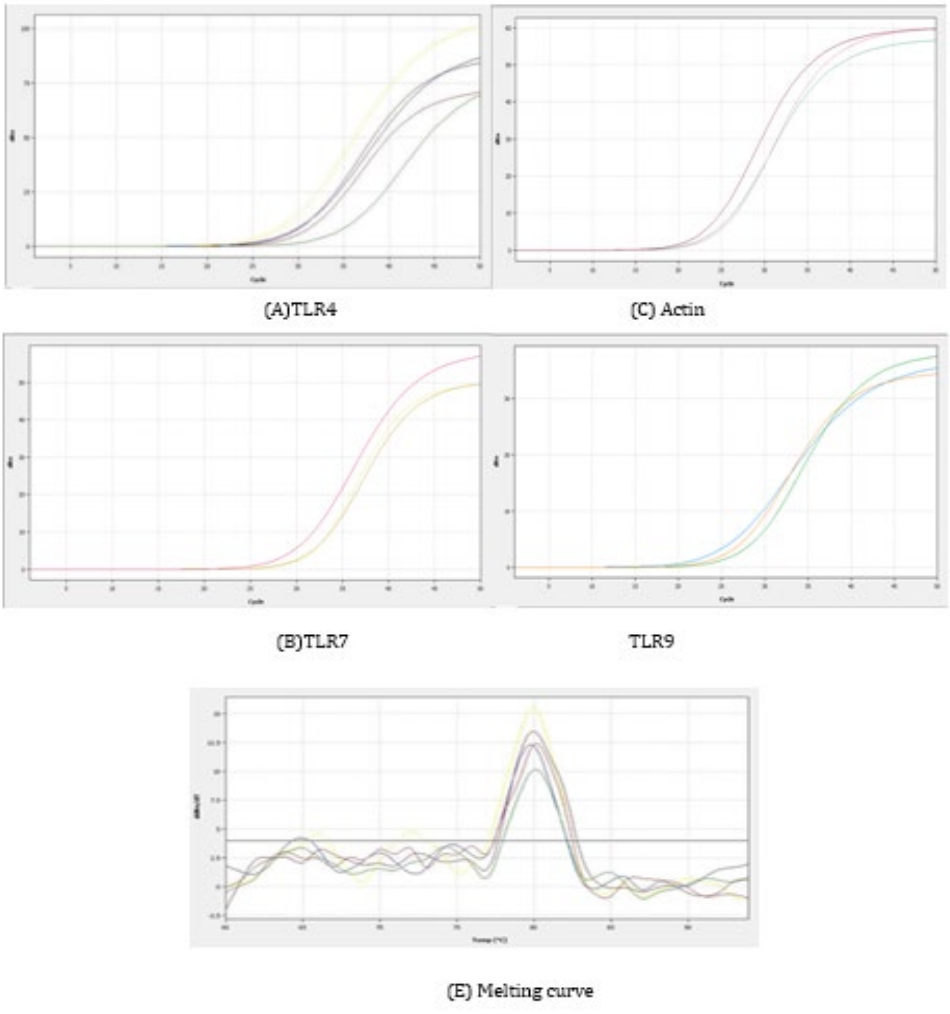

Figure 1) Bloating and melting curve of TLRs

Discussion

The most complex and life-threatening disease is cancer which affects different aspects of life [17]. It has been documented an increase in childhood leukemia (children below the age of 15 years) [18] in Iraq and specifically, Basrah, is highly populated, the area is surrounded by oil fields and subjected to being a battleground by three wars; including the Iraq-Iran war in 1980, The US invasion in 1991, and the last US invasion in 2003. These reasons might be the risks for increasing childhood leukemia [19]. Many studies were conducted to shed light on many factors that might be explained the increasing incidence, risks factors, and immune-pathogenesis of the diseases [18, 0-21]. Consequently, in this study, the authors try to investigate any possible association of gene expression of specific cell receptors with the pathogenesis of leukemia.

Cancer cells acquire immune cell characteristics, allowing them to control the immune response to benefit their development and survival [22]. The connection between B cell precursor (BCP) ALL and childhood infections was postulated decades ago, but the pathogenic processes are still controversial. Pathogens' immediate transformation of B cell precursors is improbable. Instead, immune cells that have been activated and enlarged in response to those infections may interact positively with B cell precursors, promoting leukemia. The cells are ideal candidates for such leukemia-supportive immune cells because of their involvement in the immune system and existence in the bone marrow. Multiple TLRs are expressed in both normal HSCs (Hematopoietic Stem Cells) and bone marrow niche stromal cells. Although TLR signaling may be required for the formation of a normal immune response, evidence indicates a link between increased TLR expression and signaling and hematological diseases and malignancies such as lymphoid neoplasms [23].

According to previous research TLRs are expressed by different hematological malignant cells, including ALL cells. TLR4 and TLR9 could orchestrate a signal that helps cancer cells to bypass the immune responses by increasing the expression of immunosuppressive cytokines and anti-apoptosis proteins [24]. TLR4 expression was shown to be deeply linked to tumor invasiveness [25].

There is controversy about TRL4 expression in acute leukemia, as some studies showed over-expression [26-27], while the results of Sánchez-Cuaxospa *et al.* [23] and Pehlivan *et al.* [28] demonstrated lower expression levels compared to normal control. In this study, TLR4 expression is higher in ALL patients (newly diagnosed and relapsed) compared to the control group (4.0618±2.16754, 11.3295±1.45013) respectively with $p<0.001$.

TLR4 is linked to IL10 production as an anti-TLR4 antibody can cause blockade in IL10 production. It is well-known as an immunosuppressive cytokine with a possible role in the pathogenesis and progression as higher levels in cancer patients are associated with a worse prognosis [29]. Increased IL10 production can lead to inhibition of CD8+ T cell-dependent antitumor immunity in the tumor microenvironment [30]. In solid tumors, TLR4 over-expression is associated with carcinogenesis and early metastasis according to Morais *et al.* [31]. Cytoplasmic TLR4 was shown to be strongly associated with greater tumor grade, found that TLR4 expression is greater in initial tumors than in local recurrent cancers.

The present study demonstrated the mean ΔCt±SD level of TLR-7 expression in ALL patients (-5.2200±3.05991) and in normal control (10.0243±1.38014), with a significant difference ($p<0.001$). Also, the mean Δct level of the newly diagnosed patient compared with relapsed ones (-5.11048, -5.45000) respectively, with no significant difference ($p=0.686$).

As shown previously the expression of TLR7 was high mean ΔCt value in relapsed patients than in newly diagnosed ones, this is differently supporting the role of worse disease prognosis and progress. TLR-7 appears to play a dual or contentious function in cancer formation, according to a large study [32]. The present study confirmed the finding of most authors who demonstrated high expression of TLR7 in a patient with ALL in comparison with normal control. It has been also indicated that the co-stimulation of TLR7 could induce chemo-resistance in human lung cancer cells [33].

Acute Myeloid Leukemia (AML) researchers investigated the quantitative expression of TLRs in patients with newly diagnosed or recurrent AML and determined that TLR-7 had the greatest level of TLR expression in patients than the control [34-35]. This finding supported that TLR-7 may play a role in the immune escape from acute leukemias (AML and ALL). Han *et al.* [36] found that Patients who exhibited very high TLR7 expression in tumor cells of oral squamous cell carcinoma had poor differentiation and prognosis.

These findings suggest that TLR-7 targeting may inhibit the growth and induce apoptosis of leukemic cells, providing new insights into the biology and therapy of acute leukemias. Other studies done by Basith *et al.,* [37] and Rybka *et al.* [38] found that the level of TLR7/β-actin mRNA expression was elevated in AML patients compared to normal cases with statistically significant ($p<0.05$).

Helminen *et al.* [32] discovered that TLR7 expression is increased in both epithelial and stromal compartments of human pancreatic cancer, leading them to hypothesize that TLR7 signaling is essential for carcinogenesis. Only one result is inconsistent with our finding and belongs to Sánchez-Cuaxospa *et al.* [23] that showed a low level of TLR7 in a patient with a newly diagnosed patient with ALL compared

with the normal control group. TLR expression may differ depending on the stimulus, environment, cell type, and subset, and probably age group. The TLR expression profile appears to be also influenced by the location of the cells [23].

TLR9 has heterogeneity in signaling on B cells and its expression in B-cell malignancies may influence the degree of TLR9-mediated B-cell activation [23]. In addition, the research found that neonatal naive B cells express more TLR9 than adult naive B cells [39].

The Present study demonstrates the high level of TLR9 in newly diagnosed patients with ALL. The mean ΔCt was found to be in newly diagnosed cases (4.3168±1.17849) compared with the mean ΔCt (10.9862±1.43895) in the control group. So, there was a statistically significant difference in TLR-9 mRNA expression levels between these two groups with $p<0.001$. The mean ΔCt is slightly higher in new cases than the relapsed cases (4.22905±1.00882 and 4.5010±1.48662) respectively. These findings were consistent with prior research by Ignatz-Hoover *et al.* [40] who showed high expression of TLR9 in leukemic newly diagnosed patients.

Alharbi *et al.* [41] showed that increased TLR9 expression inside cervical cancer cells may be directly related to tumor invasion and carcinogenesis by inhibiting TLR9 expressing immune cells from detecting tumor/viral antigen, therefore manipulating the immune response to malignancies. Furthermore, Smith *et al.* [42] and indicated that TLR9 has a role in tumor resumption mediated by MyD88/NF-B activation, which promotes IL-6 production. TLR9 also promotes Jak/STAT3 signaling, which controls tumor-promoting inflammation and angiogenesis [43]. Although TLR9 gene expression is identical in Memory B cell-related marginal zone lymphomas and diffuse large B cell lymphomas (DLBCLs), they have varied responses to TLR9 activation [44-45].

According to Sánchez-Cuaxospa *et al.* [23], TLR9 expression in PBMCs from pediatric patients with ALL was low when compared to those from healthy children. TLRs are expressed at varying levels in people with neoplasia. The patients' ages likely contribute to these disparities because age is a significant factor in the relationship between innate and acquired abilities adaptive immunological responses in healthy and diseased people. There is a lot of variation amongst different types of people. Reports on the research of patients and cell lines; in certain situations, it may be linked to disease activity. Similar but not identical findings from Morsi *et al.,* [35] demonstrated no statistically significant difference between the mean level of TLR-9 mRNA expression in AML patients and that in normal cases. In another study, patients with AML expressed TLR9 in monocyte-derived DCs, and has been found that TLR9 was expressed at a lower level in the patient group [26]. The results of the current study suggest that at the presentation of ALL patients, leukemic cells exposure the TLRs expression to escape immune surveillance by providing wrong signaling in the wrong direction. The more the ability to display the receptors, the worse the clinical outcome [36].

We were unable to collect a larger number of samples due to the limited time for the study. Intracellular and surface membrane expression of these TLRs has not been attempted due to limited access to required material and equipment.

Conclusion

TLR4, TLR7, and TLR9 gene expression are higher in ALL patients, whether newly diagnosed or relapsed than in the control group. TLRs expression might be part of the immune-evasion mechanism developed by the malignant cells that play an important role in leukemogenicity and disease progression.

Acknowledgments: The authors would like to thank staff members of Basrah Children's Hospital and the Children's parents for their kind help.

Ethical Permissions: The approval of the research's Ethical Committee of the College of Medicine/University of Basrah and Basra Health Authority was obtained before conducting this study at Basrah Children Specialty Hospital. Written consents were taken from the parents, or at least one of them, for patients' participation in the study to enroll their children in this study.

Conflicts of Interests: The authors declare no conflicts of interests.

Authors' Contributions: Mustafa R.A. (First Author), Introduction Writer/Main Researcher (40%); Jasim H.A. (Second Author), Methodologist/Statistical Analyst (40%); Ali Al-Salait S.Kh. (Third Author), Discussion Writer/Methodologist (20%).

Funding/Sources: None declared.

References

1- Chennamadhavuni A, Lyengar V, Shimanovsky A. Leukemia. Unknown city: StatPearls; 2021.

2- Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.

3- Hansen BA, Wendelbo Ø, Bruserud Ø, Hemsing AL, Mosevoll KA, Reikvam H. Febrile neutropenia in acute leukemia. Epidemiology, etiology, pathophysiology and treatment. Mediterr J Hematol Infect Dis. 2020;12(1):e2020009.

4- Montaño A, Forero-Castro M, Marchena-Mendoza D, Benito R, Hernández-Rivas JM. New challenges in targeting signaling pathways in acute lymphoblastic leukemia by NGS approaches: An update. Cancers (Basel). 2018;10(4):110.

5- Kakaje A, Alhalabi MM, Ghareeb A, Karam B, Mansour B, Zahra B, et al. Rates and trends of childhood acute lymphocytic leukaemia: an epidemiology study. Sci Rep. 2020;10(1):6756.

6- Khan M, Morshed AA, Ahmed TU, Khan HH, Ahmed AF, Roy S. Platelet indices as markers for remission in ALL during induction of remission: an experience of 52 cases. Bangladesh J Child Health. 2020;44(1):34-9.

7- Badr ZA, AL-Moosawi WN, Ali SK. Evaluation of VEGF-A in relation to childhood acute lymphocytic leukemia in Basrah, Iraq. Iraqi Natl J Med. 202;13(1).
8- Mustafa RA, Jasim HA, Al-Salait SKA. Quantitative determination of serum level of TLR4, TLR7 and TLR9 in pediatric acute lymphoblastic leukemia (ALL) patients in Basrah, Iraq. Biomed Pharmacol J. 2021;14(4).
9- Möricke A, Zimmermann M, Reiter A, Gadner H, Odenwald E, Harbott J, et al. Prognostic impact of age in children and adolescents with acute lymphoblastic leukemia: data from the trials ALL-BFM 86, 90, and 95. Klin Padiatr. 2005;217(6):310-20.
10- Forestier E, Schmiegelow K, Nordic Society of Paediatric Haematology and Oncology NOPHO. The incidence peaks of the childhood acute leukemias reflect specific cytogenetic aberrations. J Pediatr Hematol Oncol. 2006;28(8):486-95.
11- Sameer AS, Nissar S. Toll-like receptors (TLRs):structure, functions, signaling, and role of their polymorphisms in colorectal cancer susceptibility. BioMed Res Int. 2021;2021:1157023.
12- Patra MC, Choi S. Recent progress in the development of toll-like receptor (TLR) antagonists. Expert Opin Ther Pat. 2016;26(6):719-30.
13- Kagan J. Signaling organelles of the innate immune system. Cell. 2012;151(6):1168-78.
14- Brenner AK, Bruserud Ø. Functional toll-like receptors (TLRs) are expressed by a majority of primary human acute myeloid leukemia cells and inducibility of the TLR signaling pathway is associated with a more favorable phenotype. Cancers (Basel). 2019;11(7):973.
15- Sheyhidin I, Nabi G, Hasim A, Zang RP, Ainiwaer J, Ma H, et al. Overexpression of TLR3, TLR4, TLR7 and TLR9 in esophageal squamous cell carcinoma. World J Gastroenterol. 2011;17(32):3745-51
16- Khodadadi A, Razmkhah M, Eskandari AR, Hosseini A, Habibagahi M, Ghaderi A, et al. IL-23/IL-27 Ratio in Peripheral Blood of Patients with Breast Cancer. Iran J Med Sci. 2014;39(4):350-6.
17- Jibb LA, Croal L, Wang J, Yuan C, Foster J, Cheung V, et al. Children's experiences of Cancer care: A systematic review and thematic synthesis of qualitative studies. Oncol Nur Forum. 2018;45(4):527-44.
18- Karim Z, Khidhir KG, Ahmed RA, Hassan HA, Karim DO. Leukemia study in Sulaymaniyah Province, Kurdistan, Iraq. Chin Med J. 2016;129(2):244-5.
19- Nodell B. Childhood leukemia rates climb in southern Iraq. Unknown city: UW News; 2010 [Cited 2022 Mar 10]. Available from: https://www.washington.edu/news/2010/02/25/childhood-leukemia-rates-climb-in-southern-iraq/.
20- Hagopian A, Lafta R, Hassan J, Davis S, Mirick D, Takaro T. Trends in childhood leukemia in Basrah, Iraq, 1993-2007. Am J Pub Heal. 2010;100(6):1081-7.
21- Barrett AJ. Acute myeloid leukaemia and the immune system: implications for immunotherapy. Br J Haematol. 2020;188(1):147-58.
22- Alzahrani B. The biology of toll-like receptor 9 and its role in cancer. Crit Rev Eukaryot Gene Expr. 2020;30(5):457-74.
23- Sanchez-Cuaxospa M, Contreras-Ramos A, Perez-Figueroa E, MedinaSanson A, Jimenez-Hernandez E, Torres-Nava JR. Low expression of Toll-like receptors in peripheral blood mononuclear cells of pediatric patients with acute lymphocytic leukemia. Int J Oncol. 2016;49(2):675-81.
24- Mokhtari Y, Pourbagheri- Sigaroodi A, Zafari P, Bagheri N, Ghaffari S, et al. Toll-like receptors (TLRs): An old family of immune receptors with a new face in cancer pathogenesis. 2021;25(2):639-51.
25- Ruan M, Thorn K, Liu S, Li S, Yang W, Zhang C. The secretion of IL-6 by CpG-ODN-treated cancer cells promotes T-cell immune responses partly through the TLR-9/AP-1 pathway in oral squamous cell carcinoma. Int J Oncol. 2014;44(6):2103-10.
26- Schmitt A, Li L, Giannopoulos K, Greiner J, Reinhardt P, Wiesneth M. Quantitative expression of Toll-like receptor-2, -4, and -9 in dendritic cells generated from blasts of patients with acute myeloid leukemia. Transfusion. 2008;48:861-70.
27- Wang JQ, Jeelall YS, Ferguson LL, Horikawa K. Toll-like receptors and cancer: MYD88 mutation and inflammation. Front Immunol. 2014;5:367.
28- Pehlivan M, Sahin H, Ozdilli K, Onay H, Ozcan A, Ozkinay F. Gene Polymorphisms and Febrile Neutropenia in Acute Leukemia—No Association with IL-4, CCR-5, IL-1RA, but the MBL-2, ACE, and TLR-4 Are Associated with the Disease in Turkish Patients: A Preliminary Study. Genet Test Mol Biomarks. 2014;18(7).
29- Chan SL, Mo F, Wong SC, Hui EP, Loong HH, Mok T. The significance of serum interleukin-10 on the outcome of unresectable hepatocellular carcinoma (HCC). J Clin Oncol. 2011;29(4):205.
30- Park IA, Heo SH, Song IH, Kim YA, Park HS, Bang WSN. Endoplasmic reticulum stress induces secretion of high-mobility group proteins and is associated with tumor-infiltrating lymphocytes in triplenegative breast cancer. Oncotarget. 2016;7(37):59957-64.
31- Morais CA, de Rosso VV, Estadella D, Pisani LP. Anthocyanins as inflammatory modulators and the role of the gut microbiota. J Nutr Biochem. 2016;33:1-7.
32- Helminen O, Huhta H, Lehenkari PP, Saarnio J, Karttunen TJ, Kauppila JH. Nucleic acid-sensing toll-like receptors 3, 7 and 8 in esophageal epithelium, barrett's esophagus, dysplasia and adenocarcinoma. Oncoimmunology. 2016;5(5):e1127495.
33- Cherfils-Vicini J, Platonova S, Gillard M, Laurans L, Validire P, Caliandro R, et al. Triggering of TLR7 and TLR8 expressed by human lung cancer cells induces cell survival and chemoresistance. J Clin Invest. 2010;120(4):1285-97.
34- Webb RN, Cruse JM, Lewis RE. Differential cytokine and Toll-like receptor expression in leukemia. Exp Mol Pathol. 2007;83(3):464-70.
35- Morsi M, Gharabawy M, Hamed N, El Sawy M, Abou Seada N, Hashad R. Quantitative expression of toll-like receptors TLR-7 and TLR-9 on peripheral blood mononuclear cells in leukemias. J Hematol. 2016;5(1):17-24.
36- Han S, Xu W, Wang Z, Qi X, Wang Y, Ni Y. Crosstalk between the HIF-1 and Toll-like receptor/ nuclear factor-κB pathways in the oral squamous cell carcinoma microenvironment. Oncotarget. 2016;7:37773-89.
37- Basith S, Manavalan B, Yoo TH, Kim SG, Choi S. Roles of toll-like receptors in cancer: A double-edged sword for defense and offense. Arch Pharm Res. 2012;35(8):1297-316.
38- Rybka J, Butrym A, Wrobel T, Jazwiec B, Stefanko E, Dobrzynska O, et al. The expression of Toll like receptors and development of severe sepsis in patients with acute myeloid leukemias after induction chemotherapy. Med Oncol. 2014;31(12):319.
39- Pettengill MA, van Haren SD, Li N, Dowling DJ,

Bergelson I, Jans J. Distinct TLR-mediated cytokine production and immunoglobulin secretion in human newborn naive B cells. Innate Immun. 2016;22(6):433-43.
40- Ignatz-Hoover JJ, Wang H, Moreton SA, Chakrabarti A, Agarwal MK, Sun K, et al. The role of TLR8 signaling in acute myeloid leukemia differentiation. Leukemia. 2015;29(4):918-26.
41- Alharbi A, Alshammari W, Alreshidi T. Expressional correlation of Toll-like Receptor 9 (TLR9) with angiogenic factors and anti-apoptotic markers in cervical cancer cells. AIMS Med Sci. 2021;8(1):11-22.
42- Smith TJ, Yamamoto K, Kurata M, Yukimori A, Suzuki S, Umeda S. Differential expression of Toll-like receptors in follicular lymphoma, diffuse large B-cell lymphoma and peripheral T-cell lymphoma. Exp Mol Pathol. 2010;89(3):284-90.
43- Gao C, Kozlowska A, Nechaev S, Li H, Zhang Q, Hossain DMS, et al. TLR9 signaling in the tumor microenvironment initiates cancer recurrence after radiotherapy. Cancer Res. 2013;73(24):7211-21.
44- Guerrier T, Youinou P, Pers JO, Jamin C. TLR9 drives the development of transitional B cells towards the marginal zone pathway and promotes autoimmunity. J Autoimmun. 2012;39(3):173-9.
45- Bai L, Chen W, Chen J, Li W, Zhou L, Niu C, et al. Heterogeneity of Toll-like receptor 9 signaling in B cell malignancies and its potential therapeutic application. J Transl Med. 2017;15(1):51.
46- Al-Kahiry WM, Dammag EA, Abdelsalam HS, Fadlallah HK, Owaist MS. Toll-like receptor 9 negatively related to clinical outcome of AML patients. J Egypt Natl Can Inst. 2020;32: 15.