Journal of Vessels and Circulation
Qom University of Medical Sciences
Spring 2021. Volume 2. Number 2

Research Paper

Plasma Cell-free DNA Levels in Children With Acute Lymphoblastic Leukemia

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Please cite this article as Saberi A, Elahi A, Jalali MT, Latifi SM. Plasma Cell-free DNA Levels in Children With Acute Lymphoblastic Leukemia. Journal of Vessels and Circulation. 2021; 2 (2):63-68. http://dx.doi.org/10.32598/JVC.2.2.54.1

http://dx.doi.org/10.32598/JVC.2.2.54.1

Article info:
Received: 03 May 2021
Accepted: 02 Oct 2021
Publish: 01 Apr 2021

Keywords:
Cell free, Nucleic acids, Precusor cell, Lymphoblastic Leukemia, Lymphoma, Biomakers

ABSTRACT

Background and Aim: Plasma levels of cell-free DNA (cfDNA) are elevated in various clinical conditions, including cancer, myocardial infarction, autoimmune disease, and pregnancy-associated complications. The aim of this study was to determine the level of cfDNA to assess the potential of cfDNA as a biomarker for screening and diagnosis and also its effectiveness in the treatment of patients with acute lymphoblastic leukemia (ALL).

Materials and Methods: Overall, 40 individuals (20 healthy volunteers and 20 patients with ALL) were examined. For quantitative analysis of cfDNA, plasma purified DNA was subjected to real-time PCR amplification of the beta-globin gene. Quantification of cfDNA levels was performed at the time of diagnosis and after treatment with a common protocol.

Results: At diagnosis, in all samples, plasma levels of cfDNA were significantly elevated compared to those of healthy controls after treatment. The high levels of cfDNA decreased and returned to normal after treatment.

Conclusion: Data showed that despite the significant cfDNA concentration increment in patients than the control group, its detection and treatment potential should be more studied: however, it still can be a useful marker for screening of diseases, such as hematological neoplasms.

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1. Introduction

Circulating cell-free plasma DNA (cfDNA) has been detected in small amounts in healthy individuals (10-30ng/mL) [1]. Plasma cfDNA in a healthy person is probably originated from apoptosis of lymphocytes and other nucleated cells [2-4]. Several studies have reported the possibility of detecting and monitoring tumor-derived cfDNA in the plasma of cancer patients and its use in tumor detection and monitoring [5-7]. Circulating cfDNA in plasma is changed both quantitatively and qualitatively in various types of cancers, such as colorectal cancer [8], small cell lung cancer [9], head and neck malignancies [10], kidney cancer [11], and melanoma [12]. Therefore, cfDNA has attracted a great deal of attention as a relatively non-invasive, sensitive, and accurate diagnostic tool for the investigation of the malignant potential of cancer [13, 14].

Most studies have investigated cfDNA in solid tumors, and it has also been proposed that the cfDNA can be used for the evaluation of molecular abnormalities in hematological cancers [15-17] and there are only a few reports available for patients with the lymphoproliferative disease [18]. Acute lymphoblastic leukemia (ALL) is one of the most common hematologic malignancies in children [19]. Up to now, most of the diagnostic and prognostic tests have been performed using bone marrow (BM) aspiration. Therefore, new non-invasive, rapid, sensitive, and accurate diagnostic and prognostic markers are needed to improve the prognosis of ALL. The present study investigated not only the total plasma levels of cfDNA in pediatrics with acute lymphoblastic leukemia compared to a control group of healthy persons, but also the total cfDNA levels in plasma after treatment.

2. Material and Methods

Patients

Written consent forms were obtained from parents before enrolment and the Ethical Review Boards of the Ahvaz Jundishapour University of Medical Sciences reviewed the protocol for plasma collection. We examined 20 children with ALL and 20 healthy volunteers. Patients with approved ALL who were under the first chemotherapy period were included in this study and also patients with other hematologic malignancies (chronic lymphoblastic leukemia (CLL), Acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), and myelodysplastic syndromes (MDS [and Lymphoma]) were excluded. The main characteristics of the study population are shown in Table 1.

Blood sampling

For blood sampling, 10 ml of blood was withdrawn from each patient or healthy control into an EDTA tube. For the preparation of the plasma, blood samples were centrifuged at 1600g for 10 min. The supernatant was carefully removed and transferred into several fresh microtubes in order to be recentrifuged at 14000g for 12 min to avoid contamination by blood cells. The upper plasma was collected into several fresh 1.5ml tubes. The plasma samples were stored at -80°C until further processing on the day of analysis and the plasma was slowly thawed.

Table 1. Main characteristics of the study population

Characteristics		Mean±SD/No.(%)	
		Patients	Healthy Controls
Gender	Female	9(45)	10(50)
	Male	11 (55)	10(50)
Age (y) 7.344.39	≤10	14 (5.4±2.5)	5.12.96
	≥10	6 (20.4±18.0)	19 (4.7±2.5)
WBC (Mean) (×106/L)		25.16±29.51	7.1±2.5
RBC (Mean) (×109/L)		3.1±1.18	5.69±0.36
Platelet (Mean) (×106/L)		111±106	267±114

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Table 2. Association between patient characteristics and plasma cell-free DNA (mean) level

Characteristic		Mean±SD cfDNA	P
Gender	female	53.34±97	0.5
	male	132.35±17	
Age (y)	≤10	79.48±141	0.9
	≥10	122.37±144	
WBC	50x106/L	36.06±60	0.04
	≥50x106/L	151.54±178	

DNA extraction

Circulating cfDNA was purified from human plasma using Nucleospin plasma XS based on the manufacturer's instructions. Extracted DNA was eluted from the NucleoSpin XS column with 20 µl of elution buffer (Macherey-nagle GMBH & KG, Germany).

Real-time PCR

A StepOnePlus sequence detector (Applied Biosystem, foster city, CA, USA) was used to perform a real-time quantitative PCR assay. Purified DNA was quantified on the beta-globin gene, which is present in all nucleated cells of the body [20]. The primers for PCR amplification of the beta-globin gene consist of the beta-globin-354F (5'-GTGCACCTGACTCCTGAGGAGA-3') and beta-globin-455R (5'-CCTTGATACCA ACCTGCCCAG -3') and a dual labeled fluorescent PCR probe beta-globin-402T (5'-VICAAGGTGAACGTGGATGAAGTTGGTGG-TAMRA-3') [21], where TAMRA is 6-carboxytetramethylrhodamine. To inhibit probe elongation during PCR, the PCR probe contained a blocking phosphate group at the -3'end. A calibration curve was used to measure the initial amount, using four-fold serial dilutions of DNA in deionized water prepared from total DNA extracted from the k652 cell line. Each run of PCR was performed in triplicate and the average values were calculated. When subjected to serial dilutions of human genomic DNA, this real-time quantitative beta-globin PCR assay was able to detect the DNA equivalent from a single cell.

Statistical analysis

As the values of cfDNA do not follow a normal distribution, we used the Mann-Whitney test to compare plasma levels between patients and healthy controls. The Wilcoxon rank test was also used to compare the mean values of the plasma level of cfDNA and clinical features. A $P \leq 0.05$ was considered to be statistically significant.

3. Results

We measured the concentration of cfDNA in plasma samples of 20 ALL patients and 20 healthy controls using a quantitative real-time PCR for the beta-globin gene. The results revealed a wide spectrum of cfDNA concentrations, ranging from 1.08 to 473.26 ng/ml. There was a significant difference in the level of cfDNA between ALL patients and healthy controls ($P \leq 0.0001$). When ALL patients were analyzed after treatment, plasma cfDNA levels decreased and returned to normal levels. This decrease paralleled a decline in peripheral blood leukocyte counts. The patients were divided into three subtypes. Subtype L-1 (n=11), subtype L-2 (n=8), and subtype L-3 (n=1). No significant difference was found in cfDNA levels among subtypes. cfDNA concentrations in ALL patients were analyzed together with the main presenting clinical features (Table 2). We did not find any correlation between cfDNA levels and the age or sex of children with ALL. However, a significant positive correlation was observed between plasma DNA concentration and the WBC counts ($P=0.04$) in ALL patients. It can be concluded that cfDNA concentration in plasma can be a sensitive marker for screening of clinically significant conditions, such as hematologic disorders.

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4. Discussion

Recently, many studies have proposed the quantification of CfDNA for cancer screening [22]; CfDNA has been suggested as a cancer biomarker in adult solid cancers, such as colorectal, lung, and breast cancer [8, 9, 11]. It has been postulated that circulating cfDNA in the plasma of cancer patients originates largely from apoptosis and necrosis in tumor cells. In the present study, we quantified the concentration of total cfDNA in healthy individuals and children with ALL by means of real-time quantitative PCR. Our results revealed that DNA concentration in the plasma of children with ALL at initial diagnosis is significantly higher than in healthy individuals. In ALL patients, we observed an association between the number of leukocytes and plasma DNA levels (Mean±SD cfDNA=36.06±60 for the number of leukocytes ≤50x106/L and Mean±SD cfDNA=151.54±178 for the number of leukocytes ≥50x106/L). The higher plasma level of DNA returned to normal level after treatment. This decrease paralleled a decline in the number of leukocytes. Finally, the results suggested that plasma DNA concentration can be used as a non-invasive and sensitive but unspecific biomarker for screening of neoplastic disorders and the effectiveness of treatment. We did not find a significant decrease in cfDNA levels in the first few days after treatment. In contrast, Schwarz et al. reported that total plasma DNA in all analyzed patients was the highest on the third day and reduced rapidly, and reached levels comparable to those observed in healthy individuals by the fourth treatment day [16]. They hypothesized that this rapid decrease is due to interference of DNA replication on the fourth day. However, we think that cell lysis, either normal or tumor cells, is continued beyond the fourth day after chemotherapy [23].

5. Conclusion

Our results suggest that the plasma levels of cfDNA can be served as a relatively sensitive, noninvasive, and quick tool for screening and monitoring patients with neoplastic disorders, such as ALL but are still not specific for disease detection.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This work was financially supported by a research grant from the Vice-Chancellor of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (No: CMR30-14).

Authors' contributions

Methodology: Alihossein Saberi and Asghar Elahi; Data collection: Asghar Elahi; Investigation, Writing - original draft, and Writing - review & editing: All Authors; Data analysis: Seyed Mahmood Latifi.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

We acknowledge the cooperation of all patients and their parents, nurses, and physicians who contributed to the collection of samples for this study. We are thankful to Dr. Manoochehr Makvandi and Niloofar Neisi for expert technical assistance in real-time PCR.

References

[1] Dwivedi DJ, Toltl LJ, Swystun LL, Pogue J, Liaw KL, Weitz JI, et al. Prognostic utility and characterization of cell-free DNA in patients with severe sepsis. Critical Care. 2012; 16(4):R151. [DOI:10.1186/cc11466] [PMID] [PMCID]

[2] Gahan PB, Swaminathan R. Circulating nucleic acids in plasma and serum. Recent developments. Ann N Y Acad Sci. 2008; 1137:1-6. [DOI:10.1196/annals.1448.050] [PMID]

[3] Ozkaya O, Bek K, Bedir A, Açikgöz Y, Ozdemir T. Plasma cell-free DNA levels in children on peritoneal dialysis. Nephron Clin Pract. 2009; 113(4):c258-61. [DOI:10.1159/000235250] [PMID]

[4] Swarup V, Rajeswari MR. Circulating (cell-free) nucleic acids--A promising, non-invasive tool for early detection of several human diseases. FEBS Lett. 2007; 581(5):795-9. [DOI:10.1016/j.febslet.2007.01.051] [PMID]

[5] Esposito A, Bardelli A, Criscitiello C, Colombo N, Gelao L, Fumagalli L, et al. Monitoring tumor-derived cell-free DNA in patients with solid tumors: Clinical perspectives and research opportunities. Cancer Treat Rev. 2014; 40(5):648-55. [DOI:10.1016/j.ctrv.2013.10.003] [PMID]

[6] Ryan BM, Lefort F, McManus R, Daly J, Keeling PW, Weir DG, et al. A prospective study of circulating mutant KRAS2 in the serum of patients with colorectal neoplasia: Strong prognostic indicator in postoperative follow up. Gut. 2003; 52(1):101-8. [DOI:10.1136/gut.52.1.101] [PMID] [PMCID]

[7] Sorenson GD, Pribish DM, Valone FH, Memoli VA, Bzik DJ, Yao SL. Soluble normal and mutated DNA sequences from single-copy genes in human blood. Cancer Epidemiol Biomarkers Prev. 1994; 3(1):67-71. [PMID]

[8] Anker P, Lefort F, Vasioukhin V, Lyautey J, Lederrey C, Chen XQ, et al. K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer. Gastroenterology. 1997; 112:1114-20. [DOI:10.1016/S0016-5085(97)70121-5]

[9] Chen XQ, Stroun M, Magnenat JL, Nicod LP, Kurt AM, Lyautey J, et al. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med. 1996; 2(9):1033-5. [DOI:10.1038/nm0996-1033] [PMID]

[10] Nawroz H, Koch W, Anker P, Stroun M, Sidransky D. Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med. 1996; 2(9):1035-7. [DOI:10.1038/nm0996-1035] [PMID]

[11] Goessl C, Heicappell R, Münker R, Anker P, Stroun M, Krause H, et al. Microsatellite analysis of plasma DNA from patients with clear cell renal carcinoma. Cancer Res. 1998; 58(20):4728-32. [PMID]

[12] Fujiwara Y, Chi DD, Wang H, Keleman P, Morton DL, Turner R, et al. Plasma DNA microsatellites as tumor-specific markers and indicators of tumor progression in melanoma patients. Cancer Res. 1999; 59(7):1567-71. [PMID]

[13] Gautschi O, Bigosch C, Huegli B, Jermann M, Marx A, Chassé E, et al. Circulating deoxyribonucleic Acid as prognostic marker in non-small-cell lung cancer patients undergoing chemotherapy. J Clin Oncol. 2004; 22(20):4157-64. [DOI:10.1200/JCO.2004.11.123] [PMID]

[14] Tokuhisa Y, Iizuka N, Sakaida I, Moribe T, Fujita N, Miura T, et al. Circulating cell-free DNA as a predictive marker for distant metastasis of hepatitis C virus-related hepatocellular carcinoma. Br J Cancer. 2007; 97(10):1399-403. [DOI:10.1038/sj.bjc.6604034] [PMID] [PMCID]

[15] Rogers A, Joe Y, Manshouri T, Dey A, Jilani I, Giles F, et al. Relative increase in leukemia-specific DNA in peripheral blood plasma from patients with acute myeloid leukemia and myelodysplasia. Blood. 2004; 103(7):2799-801. [DOI:10.1182/blood-2003-06-1840] [PMID]

[16] Schwarz AK, Stanulla M, Cario G, Flohr T, Sutton R, Möricke A, et al. Quantification of free total plasma DNA and minimal residual disease detection in the plasma of children with acute lymphoblastic leukemia. Ann Hematol. 2009; 88(9):897-905. [DOI:10.1007/s00277-009-0698-6] [PMID]

[17] Yeh P, Dickinson M, Ftouni S, Hunter T, Sinha D, Wong SQ, et al. Molecular disease monitoring using circulating tumor DNA in myelodysplastic syndromes. Blood. 2017; 129(12):1685-90. [DOI:10.1182/blood-2016-09-740308] [PMID]

[18] Hohaus S, Giachelia M, Massini G, Mansueto G, Vannata B, Bozzoli V, et al. Cell-free circulating DNA in Hodgkin's and non-Hodgkin's lymphomas. Ann Oncol. 2009; 20(8):1408-13. [DOI:10.1093/annonc/mdp006] [PMID]

[19] Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin. 2014; 64(2):83-103. [DOI:10.3322/caac.21219] [PMID]

[20] Sozzi G, Conte D, Leon M, Ciricione R, Roz L, Ratcliffe C, et al. Quantification of free circulating DNA as a diagnostic marker in lung cancer. J Clin Oncol. 2003; 21(21):3902-8. [DOI:10.1200/JCO.2003.02.006] [PMID]

[21] Lo YM, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: Implications for noninvasive prenatal diagnosis. Am J Hum Genet. 1998; 62(4):768-75. [PMID]

[22] Fleischhacker M, Schmidt B. Circulating nucleic acids (CNAs) and cancer--a survey. Biochim Biophys Acta. 2007; 1775(1):181-232. [DOI:10.1016/j.bbcan.2006.10.001] [PMID]

[23] Ziegler A, Zangemeister-Wittke U, Stahel RA. Circulating DNA: A new diagnostic gold mine? Cancer Treat Rev. 2002; 28(5):255-71. [DOI:10.1016/S0305-7372(02)00077-4]

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