Nanomed Res J 9(2):172-179, Spring 2024

RESEARCH ARTICLE

Optimizing Exosome Enrichment: A Comparative Study in Pediatric Acute Lymphoblastic Leukemia Patients

Neda Saffari[1], Soheila Rahgozar[1*], Fikrettin Sahin[2]

[1] Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Iran

[2] Yeditepe University, Department of Genetics and Bioengineering, Atasehir, 34755 Istanbul, Turkiye

ARTICLE INFO

Article History:
Received 19 Apr 2024
Accepted 08 Jun 2024
Published 01 Jul 2024

Keywords:
Acute Lymphoblastic Leukemia
Plasma
Exosomes
Exoquick
Ultracentrifugation

ABSTRACT

Objective(s): Pediatric acute lymphoblastic leukemia (pALL) is the most prevalent neoplasm in children. pALL diagnostic process is invasive, and children need to be anesthetized for bone marrow aspiration. Exosomes are nanoparticles that reflect the status of parental cells. Given the elevated levels of exosomes in the peripheral blood of pALL patients, they can be readily extracted from blood plasma. The aim of this study was to compare the reliability of two different techniques of exosome purification in order to select the best method for clinical use in pALL.

Methods: Exosomes were isolated from plasma samples using two methods: ultracentrifugation and the ExoQuick exosome isolation (EQ) kit. The performances of these methods were compared based on the nanoparticle tracking analysis (NTA), field emission electron microscopy (FESEM), and immunoblotting assays.

Results: NTA results showed that exosome fractions extracted by the EQ kit were more concentrated and homogeneous compared with the ultracentrifugation method. Electron microscopy depicted spherical morphology for the isolated exosomes in both methods; however, the appearance of exosomes enriched by the commercial kit was more intact. Following the immunoblotting assays investigating the exosomal biomarkers, densitometry analysis showed that the exosome populations related to the EQ kit had higher concentrations and extreme purity.

Conclusions: The data demonstrated that the commercial kit exhibited superior efficacy in isolating concentrated, intact, and pure exosomes from small quantities of patients' plasma samples when compared to the standard ultracentrifugation method. According to those findings, the ExoQuick exosome segregation kit was preferred to be used in pALL diagnostic investigations.

How to cite this article
Saffari N., Rahgozar S., Sahin F. Optimizing Exosome Enrichment: A Comparative Study in Pediatric Acute Lymphoblastic Leukemia Patients . Nanomed Res J, 2024; 9(2): 172-179. DOI: 10.22034/nmrj.2024.02.006

INTRODUCTION

Pediatric acute lymphoblastic leukemia (pALL) is the most common cancer in children. Some of the currently used clinical tests are invasive and expensive or need specific equipment. The emergence of liquid biopsy research has catalyzed a surge in scholarly investigations dedicated to advancing noninvasive and highly sensitive methodologies for the diagnosis and treatment of patients. In the context of plasma-based studies, there is a mounting body of research that recognizes the importance of exosomes. Exosomes are nanoparticles secreted from all types of cells, especially malignant cells. They are introduced as specific indicator molecules mediating cell communication and important players in the horizontal transfer of genetic materials (1-3). Several studies have shown that exosomes isolated from the blood of pALL patients contain specific markers associated with leukemia cells, such as CD19, CD22, and CD58 (4-8) Others showed that exosomes derived from pALL cells carried specific microRNAs that could serve as diagnostic biomarkers for the disease(9-11).

Preanalytical procedures and technical

* Corresponding Author Email: *rahgozar@sci.ui.ac.ir*

handling may affect plasma-based studies. Among them, exosome isolation techniques are particularly important because the assay results are considered fundamental data, and any error or misinterpretation in their application may considerably affect downstream analyses (12-15).

The specific objective of this study was to compare the performance of an exosome purification commercial reagent (ExoQuick, System biosciences) for exosome enrichment versus the traditional technique of ultracentrifugation. This comparison was performed according to the results of Nanoparticle Tracking Analyzer (NTA), Field Emission Scanning Electron Microscopy (FESEM) and Western blot assay.

MATERIALS AND METHODS

Obtaining Blood

Obtaining blood was performed with full written informed parent's consent and permitted by the Ethics Committee of the University of Isfahan (ethical code: IR.UI.REC.1398.010). Peripheral blood was collected from patients with acute lymphoblastic leukemia referred to the Sayed-ol-Shohada Hospital (Isfahan, Iran). The inclusion criteria for patients were the diagnosis of pre-B-ALL at ages 1 to 14 approved by flow cytometry and the presence of over 30% blast in the bone marrow fraction. The exclusion criteria were diagnosis of Burkit lymphoma, identification of Philadelphia chromosome translocation t(9:22) (q34;q11), and impossibility of one-year follow-up due to patient non-attendance. Age-matched healthy controls were selected as controls. 3 patients and 3 controls participated in this study at the ages of 3, 4, and 8 years. After transferring samples to the laboratory, preanalytical procedures were correctly applied with caution to blood samples. Platelet-derived extracellular vesicles are released concomitantly with the coagulation cascade upon blood collection, potentially perturbing the veritable composition of exosomal cargo implicated in cancer pathogenesis. Therefore, exosomes were isolated from platelet-free plasma (PFP) through twice the centrifugation of plasma samples at 2500 g for 15 min at RT. Related plasma fractions were separated and stored in a freezer at -70°C .

Exosome isolation

Ultracentrifugation (Uc method)

After diluting 4 ml of PFP in equal volume with phosphate-buffered saline, centrifugation was done at 12000 g for 45 min at 4°C. Ultracentrifugation procedures were performed according to Thery et al. (2006) utilizing an ultracentrifuge (Beckman Coulter, Brea, CA) (18). Furthermore, a filtration method was incorporated in a sequential manner to increase the purity of the separated exosomes. The supernatant collected from the first stage and the exosome pellets were both filtered through 0.22 µm filters (Millipore, Burlington, MA). In the final step, exosomes were aliquoted in 200-ul fractions and preserved for next use at -80 °C.

EQ Kit

Exosome isolation commercial reagent (ExoQuick ™ Kit (System Biosciences, Palo Alto, CA) (Catalog number: EXOQ5A-1)) was selected for exosome purification from 500 µl PFP samples. Exosome purification was performed according to the instructions of the manufacturer with some modifications. Samples were centrifuged at 3000 g for 15 min. The Supernatant was transferred to a sterile tube and then purified using 0.22 um syringe filter. 63 µl ExoQuick solution was added to purified plasma. Samples were inverted gently and then incubated upright (without agitation) at 4°C overnight. At the end of the incubation time, the mixture was centrifuged again at 1500 g for 30 and 5 min, respectively, at room temperature to pellet the exosomes. the pellet was then solubilized in 200 µl of phosphate buffered saline. Furthermore, the process of filtration utilizing a 0.22 um syringe filter was applied to plasma samples to isolate exosome fractions while minimizing the presence of contaminating vesicles. Exosome solution was divided into 200 µl aliquots in phosphate buffered saline.

NTA

Size distribution, concentration, density, and Brownian motions of purified exosomes were disclosed using the Nanosight NS300 nanoparticle counter (Malvern Panalytical, Malvern, UK). Particles were monitored by the camera in liquid condition. A particle counter device was provided with the laser module. The ratio of dilution was identical for all samples. A total of five video captures (60 seconds) were performed for all samples in a 1 ml volume. The camera level was regulated at 16. The identical setting and detection threshold were selected. NTA software version 3.4 was employed for row data analysis.

FESEM

Field emission electron microscopy was employed to analyze droplets of exosome solutions in order to validate the size and morphology of the exosomes. Sample preparation was performed on diluted exosomes in 1 ml of phosphate buffered saline. The sample preparation procedure was executed in accordance with the manufacturer's protocol. (Brno, Czech Republic). Briefly, the copper holder was shielded with glue and aluminum foil. A droplet of diluted exosomes was located on the copper holder, then dried using a vacuum machine. Gold sputtering was performed on the dried sample, followed by taking images.

Immunoblotting

Primary antibodies, including HSP70 (as a peripheral membrane protein, 1:200) and CD9 (as a transmembrane protein,1:200), were selected in order to validate the number of exosomes. Goat anti-rabbit (1:5000) was selected as secondary antibody (from Cell signaling, Danvers, MA). RIPA lysis buffer was used for the extracting of proteins, and BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA (Catalog number: 23227) was employed in order to assess protein concentration. 6 µg exosomal protein was used for immunoblotting. The extracted proteins were separated on 4–20% gradient gels (Biorad). The PVDF membrane and Wet/Tank blotting system were employed during the blotting process. After membrane blocking and antibody incubation, protein bands were visualized using Enhanced Chemiluminescence (ECL) solutions (Immobilon, Temecula, CA). Image Lab Software 6.0.1 was utilized for bands quantification.

Statistical analysis

Error bars were calculated according to the mean ± standard error of the mean (SEM). $P < 0.05$ was taken as the significant difference.The data were the results of two independent experiments in triplicate. For comparison between groups, unpaired t or Mann-Whitney tests were used. GraphPad Prism 9.3.1 software was used for data analysis. Image J software was exploited for the quantification of data from immunoblotting pictures.

RESULTS

After performing isolation protocols related to the EQ and Uc method, 1ml of diluted samples from patient and control groups were injected to NTA device. Identical settings were subjected for all samples, then histogram plots were obtained. Plots indicated sharp and identical peaks for samples attained from the commercial kit, whereas those related to the traditional method show several peaks with the presence of large vesicles. Moreover, the size range of particles in the EQ method was

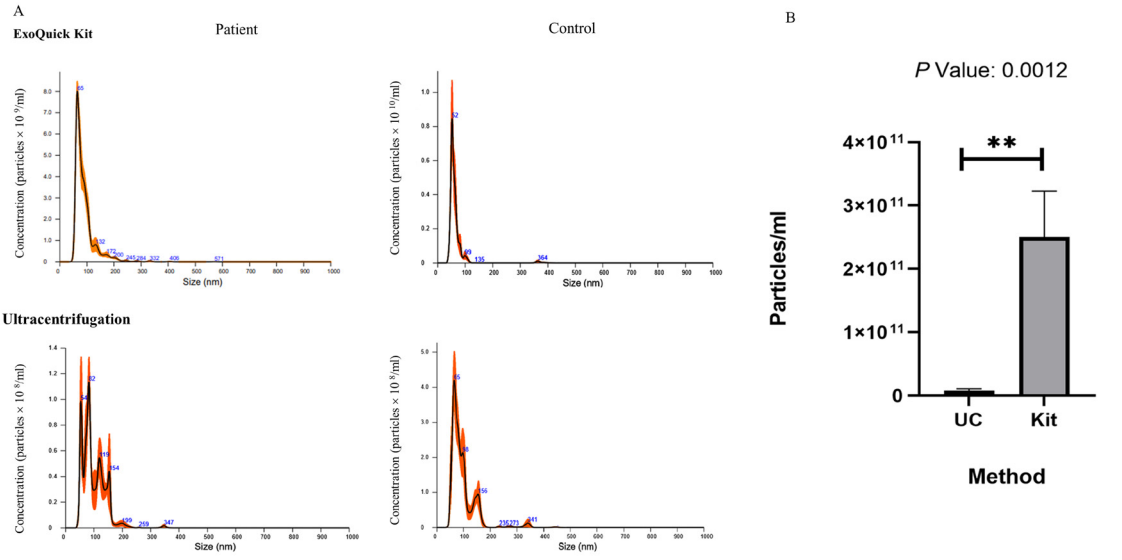

Fig. 1. Comparison between the exosome sample concentrations attained from ExoQuick kits and the Uc traditional method. A) Histogram plots illustrating NTA results indicated more concentrated samples purified from the commercial method in comparison with the ultracentrifugation method. B) Quantitative representation verified a significant difference between the exosomal yields achieved from the two different methods mentioned . Values are mean ± SEM in triplicates,** *P* value <0.005.

under 200 nm, corresponding to the actual size of exosomes (Figure 1A). Bar graph represents more particles extracted by EQ method from 1ml plasma sample compared with the Uc method (Figure 1B).

Nano sight technology evaluates fluid samples (exosomes) in natural biological condition. The representative video captures conclusively demonstrated that particles exhibiting interactions consistent with Brownian motion were identified as exosomes rather than protein aggregates or larger vesicles. It is known that heavy particles that move slowly are not exosomes. According to the results, no heavy particles were observed in samples obtained from the EQ kit. In essence, as indicated by the plots obtained through NTA analysis, there was an absence of particles detected outside the size and density range associated with extracellular vesicles, thus indicating no potential cellular contamination (19, 20). The number of particles that could be detected in a camera frame was higher for the exosome samples captured from the EQ kit compared with the Uc method (Figures

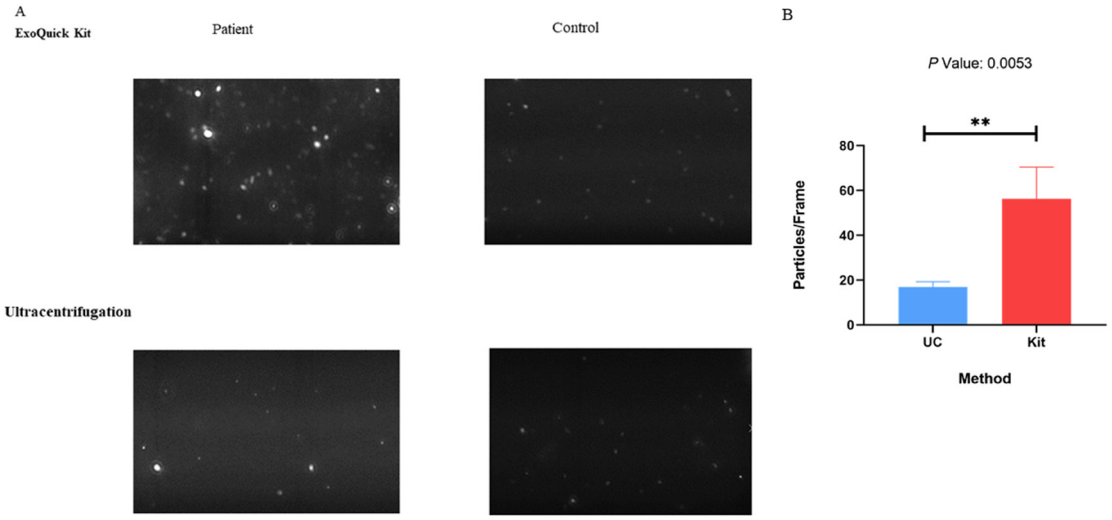

Fig. 2. Comparison between the ExoQuick kits and ultracentrifugation regarding the appearance and Brownian motions of exosomes. (A) live video captures from exosome fractions enriched by ExoQuick and the UC method. (B) The bar graph showed that the use of a commercial kit for exosomal enrichment was significantly more beneficial. Values are mean ± SEM in triplicates,** *P* value <0.005.

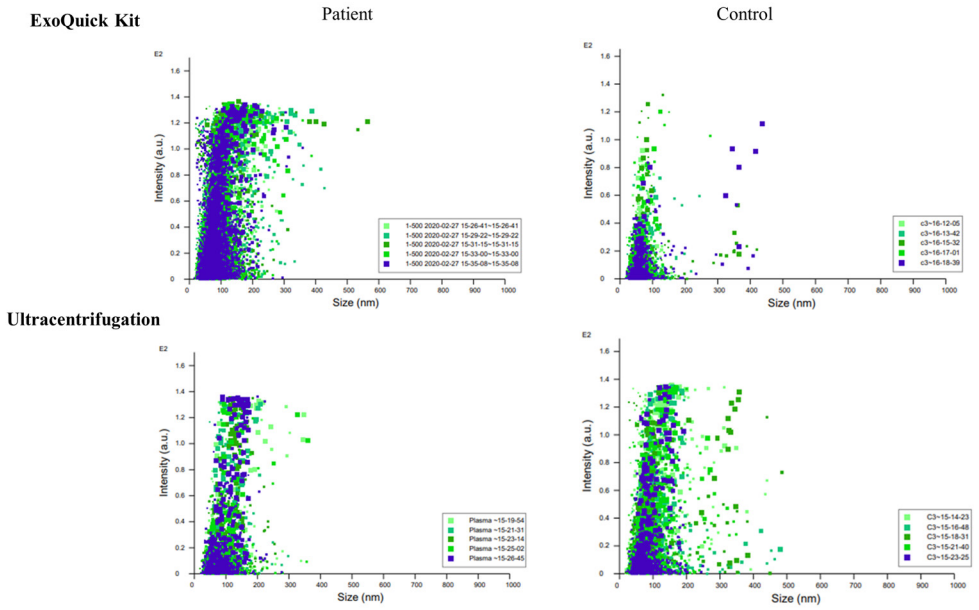

Fig. 3. Dot-plot representation of exosome samples isolated from two different methods. The dot plot showed the density of particles coincided with the density of exosomes. Furthermore, the population of particles was more homogeneous while using ExoQuick kit.

2A, 2B). The population of particles distinguished by the Uc method was heterogeneous compared with the homogenous group of exosomes purified by the EQ kit. (Figure 3).

Field emission electron microscopy demonstrated an acceptable size range of exosomes (<200 nm) extracted from both methods. FESEM confirmed the results obtained from NTA regarding the homogeneity of exosomes extracted from EQ kit compared with the Uc method. Furthermore, the spherical shape of exosomes was more evident for those attained from the commercial kit (Figure 4).

Densitometry data (Figure 5B) for Western blots (Figure 5A) was generated to compare protein abundance between samples achieved from the commercial and traditional methods. Results demonstrated that the expression levels of the exosomal markers, Hsp70 and CD9, were significantly higher for samples collected from the EQ kit compared with those acquired from the Uc method. CD9 is a member of tetraspanins; the transmembrane proteins located at the exosomal bilayer membranes (21). Hsp70 belongs to the luminal family of proteins located inside the exosomes (22).

DISCUSSION

Exosomes have been recently attracted much attention in the biological sciences, and a number of techniques have been advised for extracting exosomes from plasma or cultured media. Before performing any functional analyses followed by extraction, it is critical to assure that the purified particles are pure exosomes with no contaminating materials. Selection of the right technique for exosome extraction depends on the downstream analysis and the question of research. For instance, in the profiling studies the purity of exosomes is of great interest. In the biomarker studies, on the contrary, this is the number of exosomes which is favored. In drug delivery applications, the structure of the exosomes and their intact shape is essential.

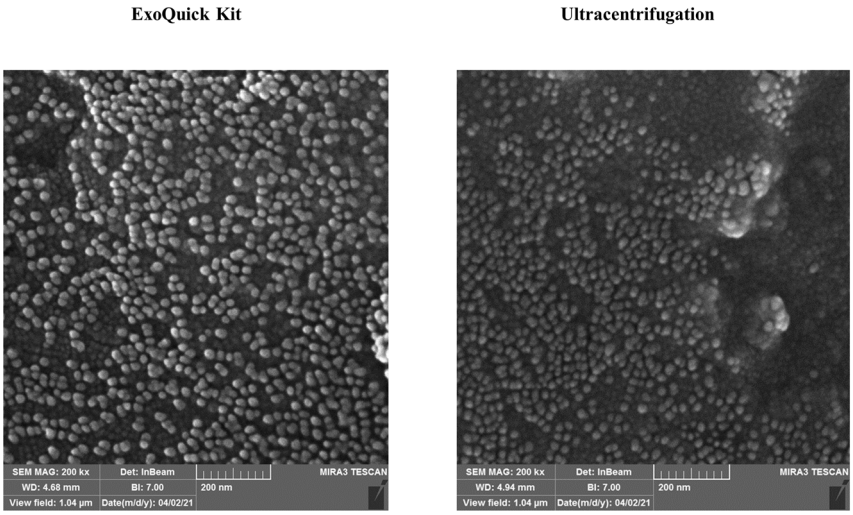

Fig. 4. Field emission scanning electron microscopy (FESEM) of exosome samples. Validation of the size and shape of exosomes. The isolated exosomal fraction using the commercial method was more concentrated. Moreover, more intact particles could be observed while using the commercial kit.

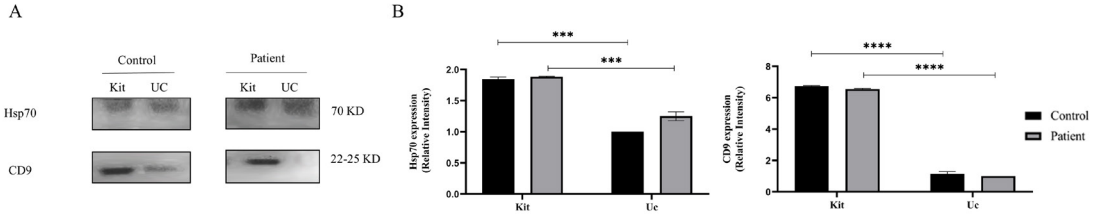

Fig. 5. Immunoblotting assay for validating the amount of exosomal marker proteins. Hsp70 and CD9 antibodies were assessed. Quantitive bar graphs indicated that exosomal enrichment was more intense while using the commercial method.***P value <0.001, **** P value <0.0001.

In other words, size, concentration, integrity and miRNA/protein content are the criteria of exosomes, attributed to the method applied for their purification (13, 14, 16, 23-25)

Over the course of previous decades, the Uc method has been widely recognized as the gold standard technique for the isolation of exosomes. This method has been widely exploited for the purification of exosomes from the culture media. There are controversies regarding the reliability of the Uc method for the extraction of exosomes from plasma or serum samples. Plasma is a highly viscous biological fluid and needs to be diluted several times before ultracentrifugation. Moreover, it contains high concentration of proteins which should be removed from plasma during or prior to exosome isolation. This procedure extends the duration of ultracentrifugation causing damage to the exosomes structure (26, 27). Exosome contamination with proteins or apoptotic bodies, which may happen through the Uc method, may cause exosomes aggregation, affecting downstream results. An additional challenge arises from the requirement for high-speed centrifuges in the Uc technique, a specialized equipment that may not be readily accessible in numerous laboratory settings according to its high price (28-32). In contrast, the EQ kit circumvents this limitation by employing standard centrifuges that are readily available in virtually every laboratory setting. The present study showed that Uc is a time-consuming method with a low-yield capacity for exosome enrichment compared to the EQ method. On the other hand, the faster Brownian motion achieved by the EQ kit implied that there was no aggregation of particles or contamination with proteins using this method (33). Results from NTA dot plot analysis and field emission scanning electron microscopy demonstrated that the extracted exosomes by the EQ kit were more homogeneous and completely intact compared with the Uc method. Moreover, the specificity of the EQ method for the isolation of exosomes was much greater than that of the traditional method (P<0.001 and P<0.0001). At last but not least, the amount of plasma samples required for the commercial kit was much less than that needed for the Uc method (500 µl vs 4 ml). In other words, the EQ kits are applicable for the tiny precious amounts of archived samples in the exosome-related investigations and may generate more and better enrichment of exosomes in these studies. Furthermore, unlike EQ kits which can accommodate a high number of samples, the operational capacity of the Uc method is constrained by the limited number of tubes that can be processed in each high-speed centrifuge run. This restriction poses challenges for scalability when dealing with a large number of samples. The groundbreaking element of this study, in contrast to other comparative investigations centered on varying exosome isolation methodologies(34) , lies in the utilization of platelet-free plasma as the sample source rather than whole plasma. Platelet-derived extracellular vesicles are released concomitantly with the coagulation cascade upon blood collection, potentially perturbing the veritable composition of exosomal cargo implicated in cancer pathogenesis. Additionally, the reproducibility of this approach was demonstrated across six distinct patient and control samples, while in similar studies only one plasma sample has been used.

CONCLUSION

Utilization of exosomes has been emerged recently as a powerful platform in medical applications for various types of cancer. Preanalytical procedures for exosome isolation are particularly of great importance, since any error or misinterpretation in their application may considerably affect downstream results. The present study was set out to determine whether ultracentrifugation, the gold standard technique known for exosome enrichment, could be substituted with a commercial method in order to be used for clinical studies in pediatric acute lymphoblastic leukemia (pALL). Results showed that even though the exosomal fractions enriched by both methods are free from contaminating aggregations, the commercial kit provides more concentrated, homogenous amounts of exosomes from 1/8th volume of patient samples in 1/3rd of the time required for the Uc method. Our findings align with those of Coughlan et al, despite the use of different assays to compare the two selected methods (35). The insights gained from our data may be of assistance to facilitate exosome sampling in pALL.

DECLARATIONS

CONFLICT OF INTERESTS

The authors affirm that they have no competing interests.

FUNDING

Authors confirmed that they have no relevant or material financial interests that relate to the research described in this paper. This project was supported by a PhD research grant from the University of Isfahan (to N.S.).

ACKNOWLEDGMENT

Authors would like to thank Dr. Alireza Moafi for his insight and kindly providing patients plasma samples, and all patients and their parents who participated in the present study.

AUTHORS' CONTRIBUTIONS

Study design: Rahgozar

Acquisition of data: Saffari

Analysis and interpretation of data: Rahgozar, Sahin, Saffari

Manuscript preparation: Saffari, Rahgozar, Sahin

Statistical analysis: Saffari

REFERENCES

1. Bartholomé R. Identification of circulation-derived exosomal microRNA candidates for liquid biopsy in pediatric acute lymphoblastic leukemia: lmu; 2022. https://doi.org/10.5282/edoc.30962
2. Fu Y, Zhang Y, Khoo BLJMRR. Liquid biopsy technologies for hematological diseases. 2021;41(1):246-74. https://doi.org/10.1002/med.21731
3. Kato M, Manabe AJPI. Treatment and biology of pediatric acute lymphoblastic leukemia. 2018;60(1):4-12. https://doi.org/10.1111/ped.13457
4. Ukrainskaya V, Musatova O, Volkov D, Osipova D, Pershin D, Moysenovich A, et al. CAR-tropic extracellular vesicles carry tumor-associated antigens and modulate CAR T cell functionality. 2023;13(1):463. https://doi.org/10.1038/s41598-023-27604-5
5. Zhu S, Xing C, Li R, Cheng Z, Deng M, Luo Y, et al. Proteomic profiling of plasma exosomes from patients with B-cell acute lymphoblastic leukemia. 2022;12(1):11975. https://doi.org/10.1038/s41598-022-16282-4
6. Zhang Y, Ge T, Huang M, Qin Y, Liu T, Mu W, et al. Extracellular vesicles expressing CD19 Antigen improve expansion and efficacy of CD19-Targeted CAR-T cells. 2023:49-63. https://doi.org/10.2147/IJN.S390720
7. Longjohn MN, Hudson J-AB, Peña-Castillo L, Cormier RP, Hannay B, Chacko S, et al. Extracellular vesicle small RNA cargo discriminates non-cancer donors from pediatric Bcell acute lymphoblastic leukemia patients. 2023;13:1272883. https://doi.org/10.3389/fonc.2023.1272883
8. Miljkovic-Licina M, Arraud N, Zahra AD, Ropraz P, Matthes TJC. Quantification and phenotypic characterization of extracellular vesicles from patients with acute myeloid and B-cell lymphoblastic leukemia. 2021;14(1):56. https://doi.org/10.3390/cancers14010056
9. Zhang J, Li S, Li L, Li M, Guo C, Yao J, et al. Exosome and exosomal microRNA: trafficking, sorting, and function. 2015;13(1):17-24. https://doi.org/10.1016/j.gpb.2015.02.001
10. Chai C, Sui K, Tang J, Yu H, Yang C, Zhang H, et al. BCR-ABL1-driven exosome-miR130b-3p-mediated gap-junction Cx43 MSC intercellular communications imply therapies of leukemic subclonal evolution. 2023;13(12):3943. https://doi.org/10.7150/thno.83178
11. Colangelo T, Panelli P, Mazzarelli F, Tamiro F, Melocchi V, De Santis E, et al. Extracellular vesicle microRNAs contribute to Notch signaling pathway in T-cell acute lymphoblastic leukemia. 2022;21(1):226. https://doi.org/10.1186/s12943-022-01698-3
12. Bæk R, Søndergaard EK, Varming K, Jørgensen MMJJoIM. The impact of various preanalytical treatments on the phenotype of small extracellular vesicles in blood analyzed by protein microarray. 2016;438:11-20. https://doi.org/10.1016/j.jim.2016.08.007
13. Salvianti F, Gelmini S, Costanza F, Mancini I, Sonnati G, Simi L, et al. The pre-analytical phase of the liquid biopsy. 2020;55:19-29. https://doi.org/10.1016/j.nbt.2019.09.006
14. Lötvall J, Hill AF, Hochberg F, Buzás EI, Di Vizio D, Gardiner C, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. Taylor & Francis; 2014. p. 26913. https://doi.org/10.3402/jev.v3.26913
15. Neerukonda SN, Egan NA, Patria J, Assakhi I, Tavlarides-Hontz P, Modla S, et al. Comparison of exosomes purified via ultracentrifugation (UC) and Total Exosome Isolation (TEI) reagent from the serum of Marek's disease virus (MDV)-vaccinated and tumor-bearing chickens. 2019;263:1-9. https://doi.org/10.1016/j.jviromet.2018.10.004
16. Witwer KW, Buzás EI, Bemis LT, Bora A, Lässer C, Lötvall J, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. 2013;2(1):20360. https://doi.org/10.3402/jev.v2i0.20360
17. Krafft C, Wilhelm K, Eremin A, Nestel S, von Bubnoff N, Schultze-Seemann W, et al. A specific spectral signature of serum and plasma-derived extracellular vesicles for cancer screening. 2017;13(3):835-41. https://doi.org/10.1016/j.nano.2016.11.016
18. Théry C, Amigorena S, Raposo G, Clayton AJCpicb. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. 2006;30(1):3.22. 1-3.. 9. https://doi.org/10.1002/0471143030.cb0322s30
19. Filipe V, Hawe A, Jiskoot WJPr. Critical evaluation of Nanoparticle Tracking Analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates. 2010;27:796-810. https://doi.org/10.1007/s11095-010-0073-2
20. Azonano W. Reviewing the use of nanoparticle tracking analysis (NTA) for nanomaterial characterization. 2015.
21. Andreu Z, Yáñez-Mó MJFii. Tetraspanins in extracellular vesicle formation and function. 2014;5:109543. https://doi.org/10.3389/fimmu.2014.00442
22. McAndrews KM, Kalluri RJMc. Mechanisms associated with biogenesis of exosomes in cancer. 2019;18(1):52. https://doi.org/10.1186/s12943-019-0963-9
23. Soares Martins T, Catita J, Martins Rosa I, AB da Cruz e Silva O, Henriques AGJPo. Exosome isolation from distinct biofluids using precipitation and column-based approaches. 2018;13(6):e0198820. https://doi.org/10.1371/

journal.pone.0198820
24. Yuana Y, Böing AN, Grootemaat AE, van der Pol E, Hau CM, Cizmar P, et al. Handling and storage of human body fluids for analysis of extracellular vesicles. 2015;4(1):29260. https://doi.org/10.3402/jev.v4.29260
25. Lacroix R, Judicone C, Poncelet P, Robert S, Arnaud L, Sampol J, et al. Impact of pre-analytical parameters on the measurement of circulating microparticles: towards standardization of protocol. 2012;10(3):437-46. https://doi.org/10.1111/j.1538-7836.2011.04610.x
26. Yu L-L, Zhu J, Liu J-X, Jiang F, Ni W-K, Qu L-S, et al. A comparison of traditional and novel methods for the separation of exosomes from human samples. 2018;2018. https://doi.org/10.1155/2018/3634563
27. Alvarez ML, Khosroheidari M, Ravi RK, DiStefano JKJKi. Comparison of protein, microRNA, and mRNA yields using different methods of urinary exosome isolation for the discovery of kidney disease biomarkers. 2012;82(9):1024-32. https://doi.org/10.1038/ki.2012.256
28. Konoshenko MY, Lekchnov EA, Vlassov AV, Laktionov PPJBri. Isolation of extracellular vesicles: general methodologies and latest trends. 2018;2018. https://doi.org/10.1155/2018/8545347
29. Yang D, Zhang W, Zhang H, Zhang F, Chen L, Ma L, et al. Progress, opportunity, and perspective on exosome isolation-efforts for efficient exosome-based theranostics. 2020;10(8):3684. https://doi.org/10.7150/thno.41580
30. Sidhom K, Obi PO, Saleem AJIJoMS. A review of exosomal isolation methods: is size exclusion chromatography the best option? 2020;21(18):6466. https://doi.org/10.3390/ijms21186466
31. Palviainen M, Saraswat M, Varga Z, Kitka D, Neuvonen M, Puhka M, et al. Extracellular vesicles from human plasma and serum are carriers of extravesicular cargo-Implications for biomarker discovery. 2020;15(8):e0236439. https://doi.org/10.1371/journal.pone.0236439
32. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. 2018;7(1):1535750. https://doi.org/10.1080/20013078.2018.1461450
33. Nazarenko IJTLB. Extracellular vesicles: Recent developments in technology and perspectives for cancer liquid biopsy. 2020:319-44. https://doi.org/10.1007/978-3-030-26439-0_17
34. Lobb RJ, Becker M, Wen Wen S, Wong CS, Wiegmans AP, Leimgruber A, et al. Optimized exosome isolation protocol for cell culture supernatant and human plasma. 2015;4(1):27031. https://doi.org/10.3402/jev.v4.27031
35. Coughlan C, Bruce KD, Burgy O, Boyd TD, Michel CR, Garcia-Perez JE, et al. Exosome isolation by ultracentrifugation and precipitation and techniques for downstream analyses. 2020;88(1):e110. https://doi.org/10.1002/cpcb.110