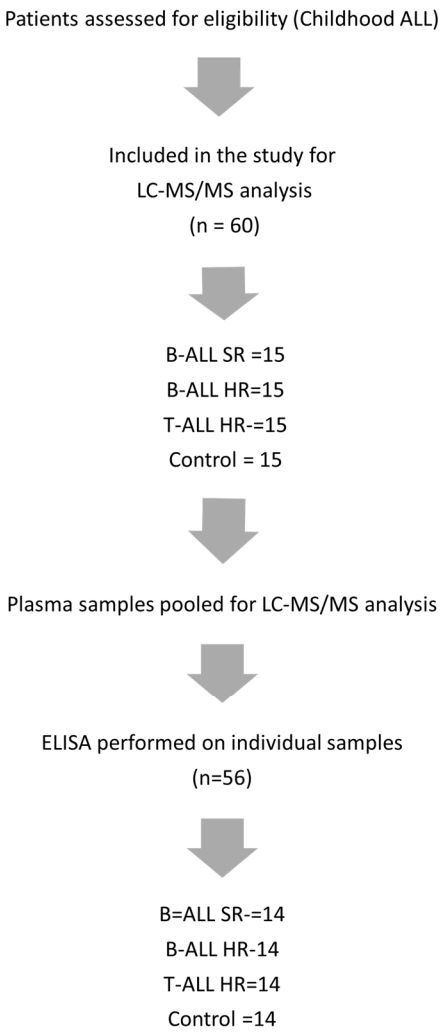

Fig S1: Flow diagram of participant selection, risk group allocation, and inclusion in analysis

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	Refer to the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1	Refer to the abstract
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	1-2	Page 1 &2, First five paragraphs of introduction
Objectives	3	State specific objectives, including any prespecified hypotheses	2	Last paragraph of the introduction "Investigating the cALL proteome …..with bioinformatics analyses"
Methods				
Study design	4	Present key elements of study design early in the paper	2	This was a case–control study conducted to evaluate the plasma proteomic profile of children with acute lymphoblastic leukaemia across different risk groups (B-ALL SR, B-ALL HR, and T-ALL HR) compared with controls. Plasma samples were analysed using LC-MS/MS for proteomic profiling, followed by ELISA-based validation of NXPE3. Statistical analysis was performed to compare protein expression across groups.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2 -3	All mentioned in materials and methods
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants		
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	2	***Healthy controls*** Children aged 1-16 years with a CBC profile within the normal range
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3	Outcomes were plasma NXPE3 levels and differentially expressed proteins; the exposure was leukemia risk group (B-ALL

				SR, B-ALL HR, T-ALL HR); predictors included protein abundance measured by LC-MS/MS and NXPE3 quantified by ELISA; potential confounders were age, gender, treatment status, and sample handling; and effect modifiers included risk group and treatment phase influencing protein expression.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	2-3	Data for proteomic profiling were obtained from plasma samples collected from children diagnosed with cALL across defined risk groups. Protein expression levels were assessed using LC-MS/MS following standardised sample preparation and pooled analysis protocols. NXPE3 concentrations were measured using a commercially available ELISA kit according to the manufacturer's instructions. Clinical and demographic variables (age, gender, and risk stratification) were obtained from patient records. To ensure comparability across groups, identical protocols for sample collection, processing, storage, and analysis were applied to all samples, and all measurements were performed under uniform laboratory conditions.
Bias	9	Describe any efforts to address potential sources of bias	N/A	
Study size	10	Explain how the study size was arrived at	2	The study size was determined based on the availability of eligible samples and the requirements of pooled proteomic analysis. To ensure equal representation and consistent dilution across groups, 15 samples were included per group (B-ALL SR, B-ALL HR, T-ALL HR, and controls), with the number limited by the smaller T-ALL cohort and the need for adequate plasma volume for LC-MS/MS and validation experiments

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	2-3	Log2 fold change values were calculated separately to assess differential protein expression between groups. Quantitative variables, including protein expression levels and NXPE3 concentrations, were summarised as mean ± standard deviation and analysed using non-parametric tests due to non-normal distribution
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	2-3	Statistical analysis was performed using non-parametric methods due to non-normal data distribution. The Kruskal–Wallis test was used to compare quantitative variables, including NXPE3 levels, across study groups. Log2 fold change values were calculated to assess differential protein expression between groups.
		(*b*) Describe any methods used to examine subgroups and interactions	N/A	
		(*c*) Explain how missing data were addressed	Only patients with a complete workup were included	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy		
		(*e*) Describe any sensitivity analyses		
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4-6	A total of 60 participants were included, comprising 45 cases (15 each of B-ALL SR, B-ALL HR, and T-ALL HR) and 15 controls. All individuals available who met the inclusion criteria during the study period were assessed for eligibility, confirmed eligible, and included in the study. As this was a case–control study using archived plasma samples, no follow-up was required, and all samples were successfully analysed.
		(b) Give reasons for non-participation at each stage	N/A	
		(c) Consider use of a flow diagram Figure S1		

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4	The study included 60 participants comprising 45 cases (B-ALL SR, B-ALL HR, and T-ALL HR; n = 15 each) and 15 controls. Demographic and clinical characteristics, including age, gender, leukaemia subtype, and risk stratification, were recorded from medical records. Exposure was defined as leukaemia risk group. Potential confounders such as age, gender, treatment status, and pre-analytical variables (including sample collection, handling, and storage conditions) were documented and considered during analysis to minimise bias.
		(b) Indicate number of participants with missing data for each variable of interest		N/A
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)		
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time		
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	4	The exposure (leukaemia risk group) was categorised into B-ALL SR (n = 15), B-ALL HR (n = 15), and T-ALL HR (n = 15) among cases, with controls (n = 15) serving as the unexposed group. Summary measures of exposure were represented by group-wise protein expression levels, including NXPE3 concentrations and log2 fold change values derived from proteomic analysis.
		Cross-sectional study—Report numbers of outcome events or summary measures		
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	4	Unadjusted estimates of NXPE3 levels were reported as mean ± standard deviation with corresponding 95% confidence intervals for each group. Differences between groups were assessed using the Kruskal–Wallis test. Due to the exploratory nature of the study and pooled proteomic design, no multivariable adjustment was performed; however, potential confounders including age, gender, treatment status, and pre-analytical variables were minimised through uniform sample selection and standardised processing across groups.
		(*b*) Report category boundaries when continuous variables were categorized	N/A	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A	
Discussion				
Key results	18	Summarise key results with reference to study objectives	Pg 4-8	Refer to the discussion
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Pg 8	Last page of discussion
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pg 4-8	Refer to the discussion
Generalisability	21	Discuss the generalisability (external validity) of the study results		The generalisability of the study findings is limited by the relatively small sample size and single-centre design. As the study population consisted of selected cases within defined risk groups of childhood acute lymphoblastic leukaemia, the results may not be fully representative of all cALL populations. Additionally, the use of pooled samples in proteomic analysis may reduce individual-level variability, potentially limiting direct clinical applicability. However, the identification and validation of NXPE3 as a potential biomarker provide a basis for further investigation in larger, multicentre cohorts to enhance external validity.
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	8	This study was partially funded by Ziauddin University, but no funding number was assigned

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.