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Importance of interpretative comments in clinical biochemistry – a practitioner's report

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ABSTRACT

Interpretative comment (IC) from the clinical biochemist is a professional obligation. Most of the Nepalese clinical laboratories use only predefined comments on the report, while few laboratories do not provide comments at all. Apart from doctors, other healthcare professionals and sometimes patients themselves seek laboratory expert opinion in the interpretation of obtained results. The non-availability of patient's medical record or limited communication with physicians as well as insufficient professional knowledge impacts the quality of interpretative comments in Nepal.

This report is intended to emphasize that the task of providing IC is becoming more important in the context of Nepal. Similarly, this report also guides those who provide interpretative comments.

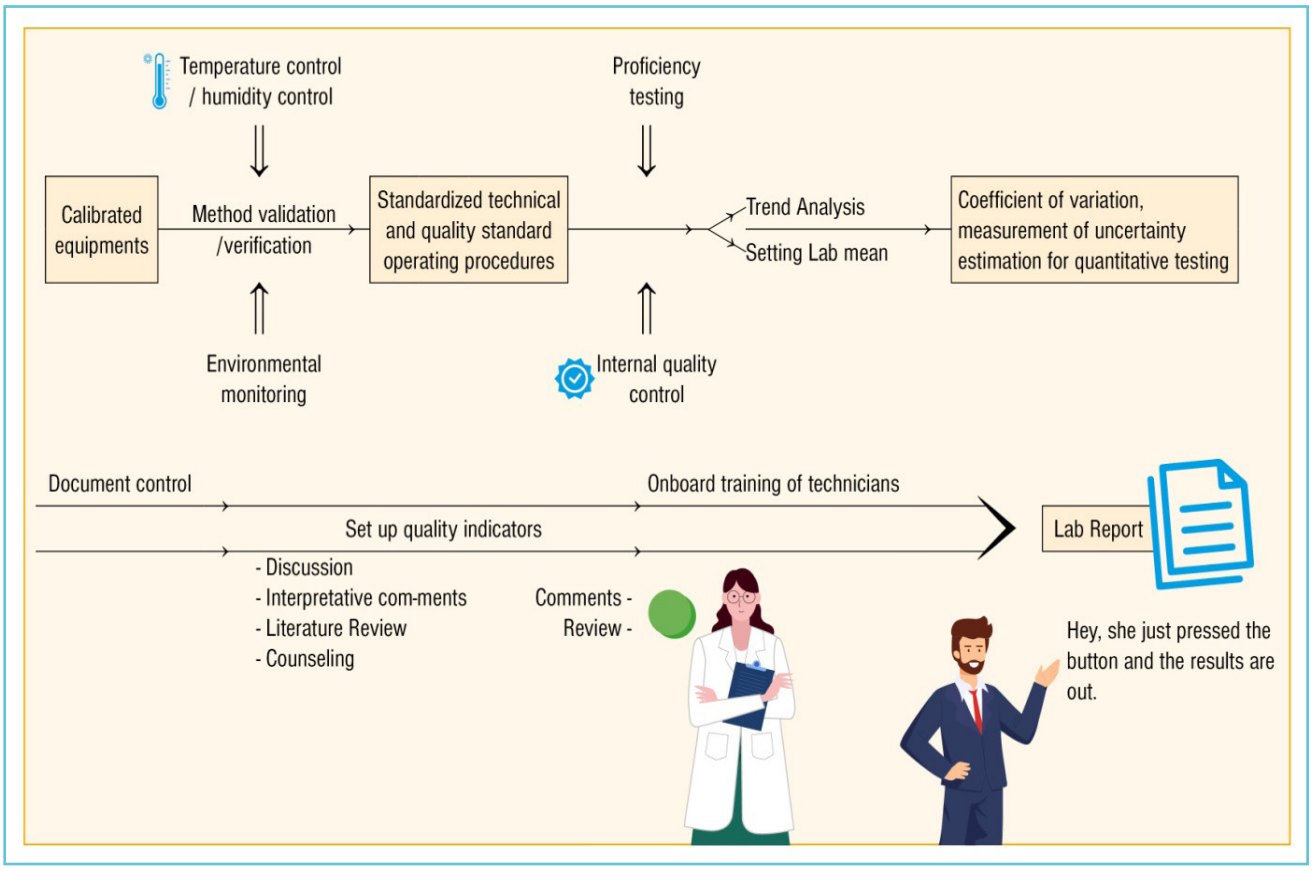
INTRODUCTION

Laboratory professionals can contribute to the test result interpretation to provide other healthcare professionals a better understanding of the obtained results. The understanding of analytical part of laboratory work and possible preanalytical influences is required for correct interpretation of test result which also requires the understanding of clinical significance of the results and patients clinical condition. Flagging up of a result outside reference interval is easily appreciated by the reader of laboratory reports, however true interpretation is based on understanding of all aspects of total testing process.

There has been tremendous improvement in the laboratory processes in the past years including preanalytical processes (such as barcoding of primary samples, electronic orders, automatic check for clots, lipemia, hemolysis and icterus), analytical processes (use of automated advanced assay platforms with low intra assay and inter assay variation) and post analytical processes (such as auto validation and connection of hospitals with laboratory information system). Together with all these improvements, the role of laboratory physician has changed which includes interaction with both clinicians and patients apart from technical chores. Clinical interpretation of obtained test result however depends on extensive practical and theoretical clinical expertise of the laboratory physician. (Figure 1)

Although there are no universal guidelines regarding the use of interpretative comments (IC), the ISO 15189 standard in its clause 5.8.3 states that the laboratory report should include

Figure 1 Role of laboratory physician in providing reports



IC where appropriate. (1) Correct interpretation of laboratory results is crucial for accurate and timely diagnosis and appropriate management of clinical condition. In two independent studies, authors reveal that IC establish a positive relationship between the laboratory and the clinic, and it reduces time to diagnosis, prevents misdiagnosis and reduces the number of irrelevant laboratory tests. (2, 3)

There is wide variation internationally, as regard to the extent to which ICs are provided on biochemistry reports. The objective of IC is to facilitate clinicians in the interpretation of complex laboratory results. This is especially important when, significant abnormalities are present, dynamic or uncommon tests are reported or where analytical or preanalytical factors not appreciated by the clinician may have influence in the interpretation of the results. In a study conducted at the laboratories in the United Kingdom, 77% of general practitioners and nurses answered that they would like to see comments on laboratory investigations. (4)

PRACTITIONER'S REPORT

When the laboratory report is incongruent to the clinical features, patients have the tendency to visit different laboratories for the same test and compare the results between laboratories. Thus, the number of patients visiting hospitals with conflicting diagnoses based on different laboratory report adds confusion to the clinician. Auto validation and electronic reporting of laboratory results is a privilege for only few laboratories in Nepal. The missing central hospital information system makes it further difficult to trace the previous laboratory reports.

Most of the biochemistry lab reports contain only computer-generated comments that are present in each report irrespective of result. Individualized narrative comments for definitive classes of tests is practiced only by a few laboratories.

This in turn is largely attributed to the lack of trained and specialized manpower in all clinical laboratories. The free flow interpretative comments are the assessment of diagnostic test result in clinical outcome which is highly dependent on clinical context. Therefore, a competent laboratory physician in all aspects of investigation, diagnosis and treatment is required for this procedure. The IC added by inexperienced laboratory professional may add danger of providing inappropriate advice in the absence of complete clinical information. On the other hand, the computer-generated comments that are by default present in each individual report helps the user to understand the basics of the test and avoids delaying the release of reports. However, these comments are very limited in their application and take no account of clinical information provided by the patient. Moreover, the textbook or internet sources on which they are based are questionable.

With the increased awareness towards evidence-based medicine, laboratories are obliged to adhere to the general requirements for quality and competence put forward by the International Organization for Standardization (ISO) 15189:2012. Few laboratories are now accreditated by this standard in Nepal that stimulates higher standards of quality within laboratories, thereby leading to more consistent and reliable test data.

There are reports of importance of ICs in diagnosing and monitoring hematological disorder and in facilitating early diagnosis of dyslipidemia associated inherited metabolic disease. (2, 5) The laboratory testing and interpretation of patients with suspected coagulation disorders requires consulting expertise and includes careful assessment of medical history, drug treatment, preanalytical and analytical factors, mixing studies and additional investigations. The situation is similar for other areas, and where ICs have significant role are morphological investigations

of peripheral smear and body fluids, electrophoretic assays, flow cytometry, toxicology and molecular tests. (6, 7)

Realizing the importance of IC in Nepalese context, adding comments for investigations was started for tests such as thyroid function, pituitary function, lipid profile, HbA1c and other test where interpretation is thought to be of help. Many comments are related to the sample quality, preanalytical interfering factors and recommendations based on the results (Table 1).

The motive of adding ICs is to help the requester to make the correct management decision for the patient, therefore it is essential that laboratory professional should contribute their best. As per the authors experience, the biochemical tests that should be considered for the possible inclusion of ICs in Nepalese context are listed in Table 2.

It should be noted that the ICs could be technical or clinical. Technical comments are related to the sample quality and preanalytical interfering factors as mentioned in Table 2 with electrolytes as an example. Similarly, clinical comments typically comprise of mentioning absence or presence of an abnormality and its severity, possible cause for unexpected result along with clinical implication and a suggested additional testing or referral. Example: Comments on manual count for platelet aggregates when a Coulter counter indicates thrombocytopenia or recommending a glycerol blank test for possibility of pseudohypertriglyceridemia in a non-lipemic sample with a very high triglyceride value. (8)

Table 1 Interpretative comments in various phases of total testing process

Phases in the total testing process	Potential sources of error	Interpretative comments on
Pre-analytical	• Inappropriate test request • Patient/specimen misidentification • Sample collected from infusion route • Sample collection (hemolysis, clotting, insufficient volume) • Inappropriate container • Improper storage and transportation • Error in sorting and routing of sample • Pour-off • Pipetting and labeling error • Error during centrifugation	• Cryoglobulins • EDTA induced platelet clumps • Icteric, hemolyzed or lipemic sample that could interfere other analytes • Physiological variation such as age, gender, pregnancy, diurnal cycle and fasting condition • Any significant context of test request

Analytical	• Equipment malfunction • Sample mix-ups • Interference (endogenous or exogenous) • Undetected failure in quality control	• Sample dilution • Results for corrected calcium/ sodium • Any changes in analytical platform
Post-analytical	• Erroneous validation of analytical data • Excessive turn-around time • Improper data entry and manual transcription error • Failure/delay in reporting critical values • Incorrect interpretation • Inappropriate/inadequate follow-up plan • Failure to order appropriate consultation	• Any changes in reference interval • Recommendation for follow up or expert consultation • Recommendation for additional investigation • Any significant change from previous result and evaluation of result from the knowledge of biological variation. • Comments regarding calculations • Interpretation of dynamic tests, coagulation test, autoimmunity test, allergy test and molecular diagnostics test.

Table 2 **Biochemical investigations that should be accompanied by interpretative comments in the Nepalese context**

S/N	Parameters	Reason for interpretative comments in the Nepalese context
1	Tumor markers	A tumor marker concentration within reference interval does not exclude malignancy. There are various causes for false positive elevation of tumor marker and also the intraindividual variation of tumor marker is high. However, these tests are included in various healthcare screening packages without proven medical benefit, designed by clinical laboratories in Nepal.
2	Electrolytes	Most of the clinical laboratories acceptance criteria for remotely collected blood sample are not well defined. There are high chances of preanalytical error (Example: improper order of draw, mislabeling of tubes, under filling of tubes, delayed centrifugation, degradation during transportation).

3	Dynamic endocrine tests	Evidence based national guidelines for dynamic endocrine tests are missing. Therefore, correct name for dynamic test along with appropriated timing of sample collection and established standard for interpretation should be mentioned in the report. The cut-offs and further recommended test based on those cut-offs should be mentioned.
4	Endocrine tests	Rechecking of results in two or more laboratories with different immunoassay platform may result in conflicting diagnosis. There is lack of knowledge among clinicians about serial dilution, hook effect, polyethylene glycol precipitation test, heterophile antibodies and biotin interference, potential interference from Ayurvedic medicine and cross-reactivity of steroidal hormones with immunoassays.
5	Autoimmunity test	There is no harmonization for sample screening dilution. The clarification for any differences in the interpretation of result obtained using different method of determination is not fully appreciated by the clinician.
6	Test run using Immunochromatography (rapid kit)	Rapid kits require little technical skill and no special equipment therefore highly variable kits are commercialized in Nepal. Alternate confirmatory method of diagnosis should be advised based on sensitivity and specificity of the diagnostic kit keeping in mind the prevalence of disease.

Scientific articles (short communication and perspectives) published in national journals to raise awareness among physicians and patients about various general routine investigations and link to these articles are mentioned along with IC for further clarification.

CONCLUSION

Interpretative comments minimize conflicting diagnosis based on different laboratory test results. Accurate and unified interpretation of test results by the laboratory personnel could be assured through active participation in external quality assurance schemes and education.

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Establishment of population specific reference intervals in healthy Pakistani adults for 21 routine and special haematology analytes

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ABSTRACT

Background

The reference interval (RI) is an interval between two limits derived from distribution of the results obtained from a sample of the reference population. These population based RIs are of paramount significance for the accurate clinical understanding of the patient's health status. Haematological RIs are heavily influenced by a variety of geographical and environmental factors. Therefore, accrediting bodies also mandate that each laboratory should establish its own RIs in its own population.

Methods

This cross-sectional study was conducted at the Department of Pathology and Laboratory Medicine, the Aga Khan University Hospital, Pakistan.

Twenty-one routine and special quantitative analytes were measured in adults aged 18-60 years who passed the initial health screening questionnaire. All samples were handled strictly following standard operating procedures. Microsoft Excel and EP Evaluator software were used for statistical analysis. Nonparametric CLSI EP28-A3C method was used to establish upper and lower confidence limits at 90% significance.

Results

A total of 323 participants passed the questionnaire and were short-listed for blood collection. There were 147 males and 176 females. Reference intervals were established in 297 participants after exclusion of 26 outliers with grossly abnormal test results. Analytes included: 8 red, and 12 white blood cell parameters, platelet count, immature platelet fraction, erythrocyte sedimentation levels, haemoglobin A and A2 levels and glucose-6-phosphatase dehydrogenase levels.

Conclusion

Routine and special haematology RIs established in this study reflect significant differences from RIs in Caucasian population. For meaningful interpretation of test results, each haematology laboratory should establish or verify RIs in the population it serves.

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BACKGROUND

As medical laboratory test data are essential for establishing health status as well as determining therapeutic management decisions, provision of accurate results is critical. The concept of a "NORMAL RANGE", coined by Grasbeck and Saris in 1969, is now termed as reference interval (RI) [1,2]. The RI is an interval between, and including, two reference limits, which are values derived from the distribution of the results obtained from a sample of the reference population [3]. This reference population is a healthy group of people for a particular age and gender. The interval represents the values found in 95% of the individuals of that group [4]. These reference population-based RIs are of paramount significance for the accurate clinical understanding of the patient's health status, and are the most commonly applied tool to determine a management protocol for the patients [5]. Haematological RIs in particular are heavily influenced by a variety of confounding factors including gender, ethnicity, geographical predisposition e.g., altitude and genetic make-up [6,7]. With this perspective in mind, the College of American Pathologists (CAP) and Clinical and Laboratory Standards Institute (CLSI) recommends that each laboratory should establish its own RI [3,8].

Since there are strict criteria for developing the RIs, it is highly unlikely for individual labs to set up their reference ranges. Therefore, the laboratories tend to collaborate and obtain the RIs from the published literature, manufacturers' package inserts, textbooks, national or international expert panel recommendations, guidelines, local expert groups or indirect approaches based on data mining [9,10]. Moreover, RIs are also subjected to various criteria that must be met beforehand—one of utmost importance being the population it is catering to should be essentially the same [11].

The Pakistani population has its own unique geographical, physical, and environmental characteristics that shape their physiology. To the best of our knowledge, there is no such study that has attempted to establish haematological RIs in Pakistani adult populations using a direct approach. These population-based RIs are expected to aid in more precise clinical decision making. In our study, we established RIs based on the population we serve and compared them to previously used RIs by our laboratory adopted from Dacie and Lewis Practical Haematology textbook [12].

METHODS

Study setting

This cross-sectional study was conducted at the Section of Haematology, Department of Pathology and Laboratory Medicine, the Aga Khan University Hospital (AKUH) in Karachi, Pakistan from August 1, to December 31, 2018. The city is the largest in the country and located at an altitude of 10 m above sea level on the coast of Arabian Sea. Approximately 90% of its inhabitants are migrants, composed of ethnolinguistic groups from all parts of the country [13]. The section of haematology serves as part of the largest public sector clinical laboratory in the country with a workload of approximately 650,000 routine and special haematology tests per annum. The laboratory adheres to the highest standards of quality and was the first to be accredited by Joint Commission International Accreditation (JCIA) and is the only one with College of American Pathologist (CAP) accreditation in Pakistan.

Haematology test menu at AKUH

The routine testing menu in haematology section includes, complete blood count (CBC), reticulocyte count, immature platelet fraction (IPF), erythrocyte sedimentation rate (ESR), malaria/filaria microscopy and body fluid cell count enumeration. Special tests include haemoglobin variant analysis by high performance liquid chromatography (HPLC), glucose-6-phosphate dehydrogenase quantification, osmotic fragility test, bone marrow examination, haemoglobin F staining by Kleihauer-Betke method, Sudan Black B, Periodic acid-Schiff and Perl's Prussian blue staining for iron. Strict QC measures are practiced at all phases of testing to ensure reliability of test results. All tests are validated before final inclusion in the testing menu. This validation process includes verification of accuracy, precision (repeatability), analytical measurement range/linearity, carry over and reference interval.

Participant recruitment

Non-probability consecutive sampling technique was used for participant recruitment. In accordance with CLSI recommendations, samples were collected from a sufficient number of qualified reference individuals to yield a minimum of 120 for analysis. A pre-designed health screening questionnaire (Table 1) was administered before collecting blood samples from participants aged between 18-60 years. The questionnaire included all pertinent attributes that provide insight into overall health of an individual. Since malaria, dengue fever, hepatitis B and C are endemic in our country, questions about these illnesses were specifically included. Participants were excluded from the analysis if one or more criteria were not fulfilled. Since red blood cell indices tend to vary in males and females, these (and white blood cells) were calculated separately in both the cohorts; all other parameters were calculated in combination (Figure 1). Serum ferritin level was also checked in all females to rule out sub-clinical iron deficiency.

Sample handling

All the quantitative tests were included for reference interval study. All pre-analytical, analytical and post-analytical standard operating procedures were followed strictly as per laboratory policy. Complete blood count analysis was performed using fully automated XN-1000™ haematology analyser (Sysmex Corporation, Kobe, Japan). Reticulocyte count and immature platelet fraction (IPF) were also done on the same analyser. Haemoglobin subtypes (Haemoglobin A and A2) were measured using BIO-RAD VARIANT™ II Haemoglobin testing system (BIO-RAD Laboratories Inc, Hercules, CA, USA). Glucose-6-phosphate dehydrogenase

Table 1 Health screening questionnaire administered before blood sample collection*

	Questionnaire	Yes	No
1	Currently taking any antibiotic/medication?		
2	Had any vaccinations within 4 weeks?		
3	Fever, cold, flu or sore throat within the last 2 weeks?		
4	Had dengue fever in past 4 weeks?		
5	Had malaria in past 1 year (any malaria species confirmed by test)?		
6	Pregnancy within 6 weeks or are you pregnant now?		
7	Had a major surgery/procedure in past 1 year?		
8	Had a blood and blood component transfusion within 12 months?		
9	Any history of jaundice in past 1 year?		
10	Tested positive for the Hepatitis B surface Antigen or Hepatitis C?		
11	Known iron deficiency or Thalassemia Minor?		
12	Any chronic disease such as hypertension, diabetes mellitus, lung disease, kidney disease, liver disease, heart disease, acquired immunodeficiency syndrome, cancer, convulsions/fits, etc.?		
13	Have you ever had bleeding condition or a blood disease?		

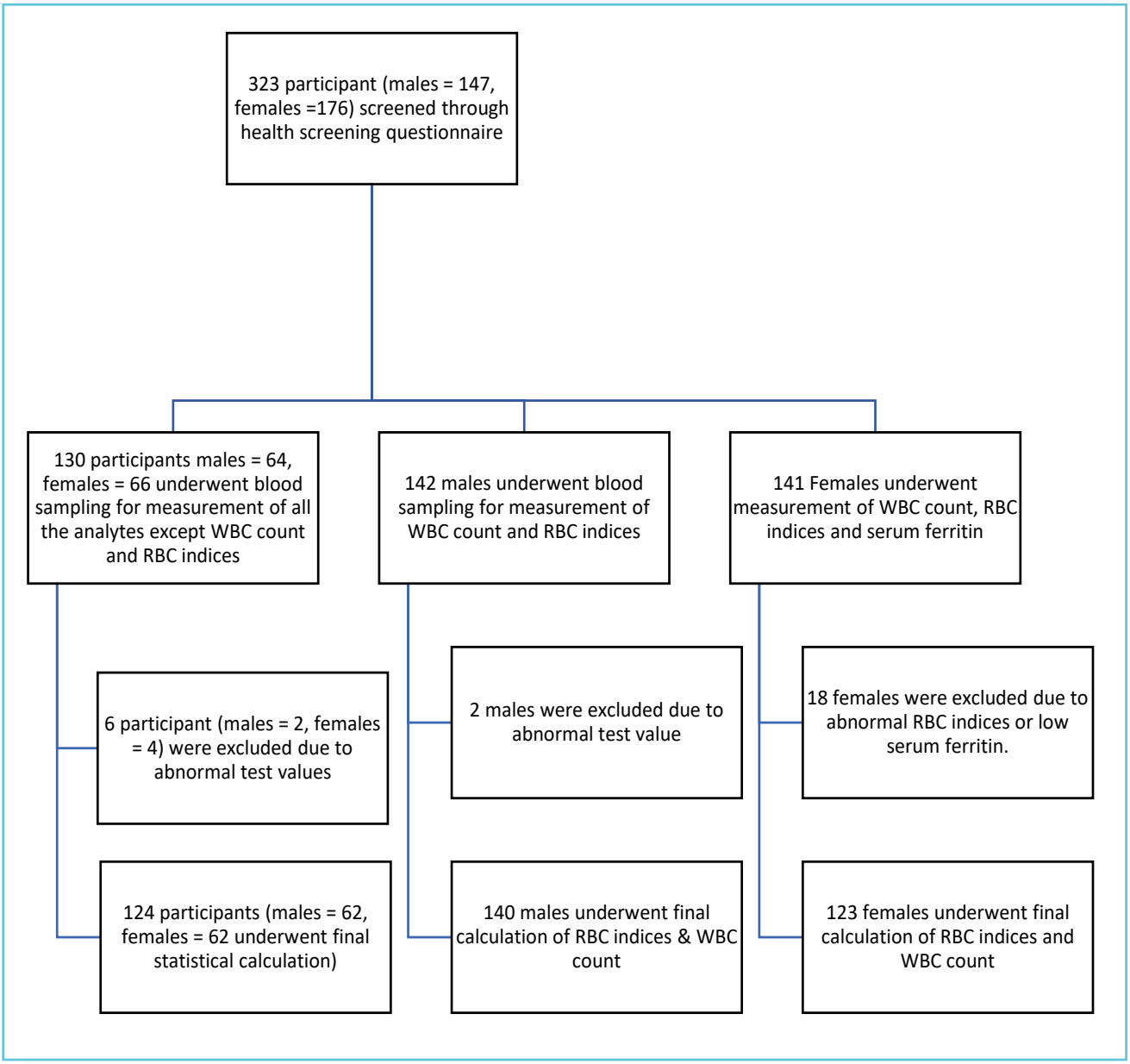
**Blood sample was collected only if the response to all of the above questions was "NO".*

(G6PD) enzyme levels were quantified spectrophotometrically using the Pointe Scientific reagent set (Pointe Scientific Inc, Canton, MI, USA). Erythrocyte sedimentation rate was measured on Sed Rate Screener 20/II (SRS 20/II; Greiner Bio-One, Kremsmünster, Austria).

Statistical analysis

Statistical analysis was done using MS Excel (Microsoft Corporation, Washington, United States) and EP Evaluator version 10.3.0.556 (Data Innovations, LLC, VT, US). Nonparametric CLSI EP28-A3C method was used to establish

Figure 1 Flow chart depicting inclusion and exclusion of participants in the study



upper and lower confidence limits at 90% significance [3].

Ethical aspects

The study was started after obtaining approval from the institutional ethical review board (5140-Pat-ERC-17). Informed consent was taken from each participant before collecting blood samples. In case abnormal test results were found, participants were appropriately guided about consulting their physicians.

RESULTS

A total of 323 participants passed the initial questionnaire (Table 1, Figure 1) and were short-listed for blood collection. There were 147 males and 176 females. One hundred and twenty-four participants underwent calculation of all the

Table 2 Instrument quality control and tolerance limits before analysis

Instrument	Parameter		Acceptable limits	Our value Acceptable yes/no
Sysmex XN-1000™	Background checks*	RBC (x 10^{12}/L)	0.02 or less	Acceptable
		Haemoglobin (g/dL)	0.1 or less	Acceptable
		WBC (x 10^{9}/L)	0.1 or less	Acceptable
		Platelets (x 10^{9}/L)	10 or less	Acceptable
	XN CHECK™ Control	Level I (Low abnormal)	#	Acceptable
		Level II (Normal)	#	Acceptable
		Level III (High abnormal)	#	Acceptable
	Pneumatic Unit Pressures (Megapascals)		0.25 ± 0.04	Acceptable
			0.16 ±0.16	Acceptable
			0.07 ± 0.01	Acceptable
	Pneumatic Unit Vacuum (Megapascals)		-0.037 – (-0.054)	Acceptable
	Aspiration Sensor Span		6500 ± 500	Acceptable
	Ambient temperature (Celsius)		15-30	Acceptable
BIO-RAD VARIANT™ II TURBO	Lyphochek® Haemoglobin A2 Control	Level I (Normal)	#	Acceptable
		Level II (High abnormal)	#	Acceptable
	Maximum Pump pressure (kg/cm^2)		<280	Acceptable
	Chromatographic station ambient operating temperature (Celsius)		15-35	Acceptable

Sed Rate Screener 20/II	Accu-Sed® Plus Control	Normal	#	Acceptable
		High abnormal	#	Acceptable
	Internal temperature (Celsius)		15-32	Acceptable
	Mechanical calibration reference		300 ± 10	Acceptable
	Delta value between reading cycles		± 2	Acceptable
Thermo Fisher Scientific GENESYS 150 UV-Vis Spectrometer	Trinity Biotech G-6-PDH Control	Normal	#	Acceptable
		Deficient	#	Acceptable
	Absorbance with water (Blank)		Zero	Acceptable
	Wavelength (nanometres)		340	Acceptable

#More than 1 control lots (with variable acceptable limits) were used during the study period.
**Analysis without aspirating the samples to verify the effects of auto rinse.*

analytes with WBC count and RBC indices, calculated separately in male and female cohorts (Figure 1, Table 3 & 4). The median age in male and female participants was 27 and 31 years, respectively.

All the quality control and instrument tolerance limits were acceptable before sample analysis (Table 2). Clinical and Laboratory Standards Institute recommends that only extreme outliers be removed—an extreme outlier being defined as one for which the distance to the adjacent value exceeds one-third of the total sample range [3]. Based on this criterion, a fraction of participants (n = 26; males = 4, females = 22) were excluded as depicted in Figure 1. Table 3 shows RBC indices in males and females. Table 4 shows white blood cell counts with differential counts in absolute numbers as well as in percentages. Table 5 shows platelet count, IPF, G6PD, ESR, haemoglobin A (HbA), haemoglobin A_2 ($HbA_{2)}$ and reticulocyte counts in the study population.

DISCUSSION

Most regulatory bodies including CAP advocate the use of population specific RIs in clinical laboratory reports to make them comprehensible and clinically useful [8]. To the best of our knowledge and belief, current study is the most comprehensive adult haematology RI data to date in Pakistan. Firstly, participant recruitment criteria were very stringent; both in terms of pre-sampling health screening of participants (Table 1) as well as monitoring of pre-analytical and analytical aspects of quality assurance (Table 2). Secondly, the study does not only include routine parameters but also advanced clinical parameters such as IPF, that has been approved later by the United States Food and Drug Administration (FDA, US) and The National Institutes of Health Clinical Centre (CC NIH) [14]. Furthermore, inclusion of specialized tests such as haemoglobin A, A2 and G6PD makes this data a complete reference package for regional haematology laboratories.

In scenarios where establishing reference intervals by including at least 120 individuals is not possible either due to cost constraints or any other reason, CLSI recommends verification of pre-existing reference interval data. This verification can be achieved using 20 samples instead of 120 samples. However, the existing reference interval data should be from a population that is as close as possible to the population being catered by the verifying laboratory. Therefore, our data can serve as database that can be utilized by other laboratories in Pakistan and in the region which intend to verify reference intervals in their laboratories.

Table 3 Red blood cell indices in study population

Analyte	Gender	Lower		Upper		Previously used RIs
		Value	95% confidence interval	Value	95% confidence interval	
RBC (x10^{12}/L)	Male	4.25	4.1-4.39	6.02	5.81-6.25	5.0± 0.5
	Female	3.61	3.6-3.9	5.2	5.09-5.40	4.3 ± 0.5
Haemoglobin (g/dL)	Male	12.3	12.0-12.7	16.6	16.2-16.8	15 ± 2.0
	Female	11.0	11.0-11.1	14.5	14.2-15.1	13.5 ± 2.0
Haematocrit (%)	Male	38.4	36.9-39.5	50.7	49.6-51.4	45 ± 5.0
	Female	34.5	33.9-34.8	45.4	44.1-47.7	41 ± 5.0
Mean cell volume (fL)	Male	78.7	73.9-80.4	96.3	94.7-98.5	92 ± 9.0
	Female	78.1	76.2-79.3	95.3	94.2-99	92 ± 9.0
Mean corpuscular haemoglobin (pG)	Male	25.1	23.7-25.5	31.6	31.1-32.6	29.5 ± 2.5
	Female	25.3	25.0-25.5	31.7	31.0-32.5	29.5 ± 2.5
Mean corpuscular haemoglobin concentration (g/dL)	Male	30.0	29.2-30.6	35.5	34.8-36.6	33 ± 1.5
	Female	30.3	30.30.4	34.4	33.9-34.6	33 ± 1.5
Red cell distribution width (%)	Male	12.0	12.0-12.1	16.0	15.3-16.5	12.8 ± 1.2
	Female	12.1	11.5-12.3	16.9	16.7-18	

Table 4 White blood cells (WBC) and differential counts in study population

Analyte		Lower		Upper		Previously used RIs
		Value	90% confidence interval	Value	90% confidence interval	
White blood cell ($x10^9$/L)	Male	4.88	4.6-5.48	11.38	10.56-12.4	4.0-10
	Female	4.6	4.0-5.09	10.8	10.2-10.9	4.0-10
Neutrophils	Absolute	1.81	1.6-2.2	7.59	6.9-7.9	2.0-7.0
	Percentage	34.9	29.4-38.7	76.2	71.3-78.8	40-80
Lymphocytes	Absolute	1.1	1.07-1.5	4.75	4.0-5.0	1.0-3.0
	Percentage	17.5	11.9-19.6	45	44.5-46.4	20-40
Monocytes	Absolute	0.20	0.14-0.2	1.0	0.9-1.1	0.2-1.0
	Percentage	3.9	1.7-4.5	10.0	9.8-10.3	2.0-10
Eosinophils	Absolute	0.02	0.0-0.04	0.6	0.51-0.79	0.02-0.5
	percentage	0.3	0.2-0.6	7.4	7.1-8.4	1.0-6.0%
Basophils	Absolute	0.01	0.0-0.01	0.09	0.08-0.10	0.02-0.1
	Percentage	0.10	0.03-0.20	1.0	1-1.2	< 1-2 %
Neutrophil Lymphocyte ratio	Ratio	1.0	0.9-1.1	4.0	3.2-4.6	#

**Absolute values are in billion cells per litre (x 10^9/L).*
 # Recently included in test menu; our own established RI is reported since the initiation of test in our laboratory.

Table 5 Platelet count, IPF, ESR, G6PD, HbA, HbA_2 and reticulocyte count in study population

Analyte	Lower		Upper		Previously used RIs
	Value	90% confidence interval	Value	90% confidence interval	
Platelet (x10^9/L)	154	142-182	433	384-488	280 ± 130
IPF (%)	1.2	0.6-1.4	8.3	7.7-9.2	#
ESR (mm/hr)	2	1-4	15	12-18	<10
G6PD (U/gHb)	6.0	5.4-6.4	12.4	11.3-13.9	8.83 ± 1.59
Haemoglobin A (%)	86.5	85.0-87.5	97.9	97.6-98.3	96.0-97.8
Haemoglobin A2 (%)	2.4	2.3-2.4	3.2	3.1-3.5	2.2-3.5
Reticulocyte Count (%)	0.6	0.4-0.7	2.4	2.1-2.5	0.5-2.5

 # Recently included in test menu; our own established RI is reported since the initiation of test in our laboratory.

Besides being the most comprehensive Pakistani data, the other significant strength of our study is utilization of appropriate statistical calculation as per CLSI guidelines. We utilized non-parametric test to establish RI. One Pakistani study reported mean ± SD values as RI which is simplest example of parametric method [15]. In this study, only routine CBC parameters were reported. Use of mean ± SD is feasible only when one is confident that the study population follows Gaussian distribution. A parametric method based on false assumption may be unreliable; not only is the estimate unreliable, the 90% confidence interval is also overly optimistic [16]. Therefore, it is not recommended to use mean ± SD unless it is very clear that the curve really is Gaussian.

As depicted in Tables 3, 4 and 5, the results obtained in this study are considerably different from the previously used RIs. For instance, MCV and MCH values obtained in current study are lower than previously used Caucasian values. It follows that should our local laboratories continue to use previously adopted values, patients with microcytic hypochromic anemia can falsely be missed and this variation can lead physicians to think about alternate diagnosis and hence wastage of important human efforts and financial resources. Similarly, upper limit of ESR in our study is 15mm (in first hour) as compared to previously used value of 10mm. Unfortunately, ESR values falling in between 10-15 mm would falsely have misled physicians to believe that their patients had some kind of

inflammatory condition. This further supplements the fact that population-specific RIs are imminently needed in the Pakistani population which has a unique genetic framework compared to Caucasian population, from which most of the currently used RIs by various laboratories are adopted.

Moreover, a noteworthy strength is gender partitioning for WBC count and red cell indices which was missing in previously used RIs. Even though, owing to the financial constraints, gender-based partitioning was not undertaken for RIs of all parameters, this fact doesn't add substantial limitation as these parameters are unlikely to vary significantly between genders. Except for red cell parameters, no significant data for other parameters were found on literature search indicating gender-based differences in RIs. Of note, total allowable error (TEa) values of most analytes other than RBC indices are significantly higher. Based on biological variations, TEa is a widely accepted concept in laboratory medicine that expresses the degree of error in a test result that can be tolerated without negatively impacting patient care. For instance, acceptable CLIA values of TEa for haemoglobin, haematocrit, red blood cells, white blood cells and platelets are 7%, 6%, 6%, 15% and 25%, respectively. This fact further supplements improbability of gender-based variation for analytes other than RBC indices. Although with significant overlap, red cell parameters in alpha thalassemia carriers might differ from normal subjects; this possibility was kept in check by monitoring fast moving peaks (before 1 minute) on high performance liquid chromatography. One limitation of our study was the lack of evaluation of dietary and environmental impacts such as sub-clinical iron deficiency. In the overall design of the study, including completely voluntary inclusion of participants, the administered health screening questionnaire did include evaluation of iron deficiency (Table 1). Additionally, serum ferritin levels were checked in all females and reactive increase in serum ferritin was ruled out by correlating with health screening questionnaire, normal white blood cell counts and erythrocyte sedimentation rates.

The confounding effect of variation in altitude is an important factor that must be addressed in RI studies. Ideally, blood samples from individuals residing in different cities of the country at different altitudes must be included in the study for comparative analysis. This was not possible in our situation principally because of cost constraints. This study was a special effort by our laboratory's management in terms of financial support. Nevertheless, we have good data at hand to start off which we consider as a first ray of light in complete darkness. In future, we intend to collaborate with laboratories situated at different altitudes in the country for measurement of the same parameters using the same reagent/equipment and study design. The samples will be added on to our laboratory's daily routine runs to remove errors arising from inter-laboratory differences linked to methodologies, operator competency and statistical compilation of the RIs. This expanded project design will produce data of vastly incremental clinical and education value not just for the people of Pakistan but also for the readership of manuscript. Future studies are required to further explore these points. Nonetheless, despite the possible highlighted short comings, the established RIs reported herein have the potential to serve as a vital resource for diagnostic laboratories in Pakistan and neighbouring regions.

CONCLUSION

Routine and special haematology RIs established in this study reflect significant differences from RIs in Caucasian populations. For meaningful interpretation of test results, each haematology

laboratory should establish or verify RIs in the population it serves.

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Ethics approval

This study was approved by Aga Khan University ethical review committee (AKU-ERC).

Consent for publication

Not applicable as this manuscript contains no individually identifiable details or images.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MSS designed the study, collected/analysed data and wrote the initial draft. AK, SA and MHH contributed to writing of the manuscript. US and NA critically reviewed the manuscript. All authors contributed to conception of the study and approved the final version of the manuscript.

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Clinical thresholds for pseudohyperkalemia and pseudonormokalemia in patients with thrombocytosis

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interference, platelet, cut-off, hyperkalemia, potassium

ABSTRACT

Background

The lysis of platelets during in vitro coagulation leads to increased potassium concentrations.

We aimed to establish the cut-off value for platelet count interfering serum potassium and to estimate the percentage of cases of pseudohyperkalemia and pseudonormokalemia in our hospital.

Materials and methods

Individuals diagnosed with essential thrombocytosis (2010-2019) based on the WHO criteria for the classification of myeloid neoplasms and acute leukemia were considered.

The cut-off value for the interference of platelet count on serum potassium results was calculated using the

reference change value. Sensitivity and specificity were calculated using a ROC-curve, and the size of the effect by the Cohen's d.

The clinical impact of both phenomena was assessed by reviewing the medical records of individuals classified as such, and also looking for potential cases in 2019 on the laboratory information system.

Results

Fifty-four individuals with essential thrombocytosis were included. Potassium concentration correlated with platelet count (P-value<0.001; Spearman's ρ =0.394) in serum. The cut-off value of platelet count interfering potassium was 598x10^3/µL [CI95%: 533–662x10^3/µL], with an associated sensitivity and specificity of 0.67 [CI95%:0.52–0.80] and 0.58 [CI95%:0.42–0.72] respectively.

The medical records of patients classified as pseudohyperkalemia or pseudonormokalemia did not include any medical action for the modification of potassium levels. In 2019, up to 0.14% of the total serum potassium determinations were susceptible to be pseudohyperkalemia or pseudonormokalemia.

Conclusion

This study provides a cut-off value for platelet count interfering serum potassium concentrations, and brings to light not only pseudohyperkalemia-related issues, but also the pseudonormokalemia phenomenon, which usually goes unnoticed.

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INTRODUCTION

Potassium ion (K^+) concentrations in plasma are kept within a narrow range thanks to its homeostatic mechanisms. The increase *in vivo* in extracellular potassium ion (K^+), known as hyperkalemia (HK) (decrease in K^+ removal by the kidney, rhabdomyolysis, tumor lysis, hemolysis, etc.) may produce neuromuscular and cardiac hyperexcitability, resulting in mild muscle cramps, weakness, paralysis or extremely severe arrhythmia.[1,2]

Pseudohyperkalemia (PHK) is a common finding in clinical samples from individuals with essential thrombocytosis (ET) or reactive thrombocytosis (RT), as a consequence of marked in vitro elevation of serum K^+ levels in the absence of clinical evidence of electrolytic imbalances. Multiple studies have reported that this elevation may be a result of the lysis of platelets or other cell components during blood coagulation.[3-5] and has been historically defined as an increase in serum K^+ concentration of 0.4 mmol/L over plasma.[6] In contrast, pseudonormokalemia (PNK) is a less known phenomenon, although resulting from the same mechanism that goes more easily unnoticed by physicians. In PNK, where K^+ values are accepted as 'normal' when the patient is actually hypokalemic and may need to be treated.

From a preanalytical point of view, it is accepted that sample collection and management are crucial for a proper assessment of K^+ levels. Some frequent causes of PHK include an excessive duration of transportation and subsequent delay in centrifugation, sample refrigeration before centrifugation, improper phlebotomy technique, sample contamination with potassium EDTA, sample transportation through pneumatic tube, hemolysis and also possible seasonal variations in environmental temperature.[7-9] When PHK or PNK is suspected, the determination of K^+ concentration is recommended either in lithium heparin (LH) plasma or in whole blood samples with balanced LH.[10] In some countries, or specialized clinical settings, lithium heparin tubes are strongly recommended to robustly assess and rule out possible cases of falsely increased potassium values. This

requires physicians and nurses to be aware of such potential situations, and prevent any delay in the diagnosis.

Despite extensive literature evidencing a positive correlation between thrombocytosis and K^+ concentration measured in serum, very few studies have tried to establish a valid cut-off value for the platelet count above which K^+ results should be interpreted with caution, let alone their clinical impact.

Hence, the aims of our study were: 1) to establish the cut-off value for platelet count in whole blood that yields K^+ variations in serum above the reference change value (RCV) and, based on these results, 2) to estimate the percentage of cases of PHK, PNK and HK in our hospital together with the clinical outcomes related to over-or undertreatment.

MATERIAL AND METHODS

Study design

This was a retrospective observational study performed at Hospital Universitari Son Espases (Palma de Mallorca, Spain), which is a tertiary care hospital giving direct service to a population of about 325,000 inhabitants. The analytical results included in the study were obtained from the laboratory information system (LIS) GestLab (Indra Cointec, Spain), and the medical records were obtained from the hospital information system Millennium (Cerner Corporation, USA), after obtaining the approval by the Ethics Board of our institution [Research Ethics Committee of the Balearic Islands (CEI-IB), nº IB 4191/20 PI].

Patients diagnosed with ET (2010-2019) based on the WHO criteria for the classification of myeloid neoplasms and acute leukemia,[11] which were appointed for a control blood examination (including complete blood count and basic metabolic panel) by the Department of Hematology were considered. Individuals were included if they had at least two control blood analyses after diagnosis date within a time frame of <4 months, both of them including platelet count, red blood cell count ($<5.8x10^6$/uL) and leukocyte count ($<20x10^3$/uL) in whole blood, and creatinine (<1.2 mg/dL; 106.1 µmol/L), potassium and hemolysis index (≤0.003 g/dL free Hb) in serum.

Another inclusion criterion was that one of both blood analyses had a platelet count between 300-$400x10^3$/µL (reference interval: 150-$400x10^3$/µL) whereas the other had an altered result ($>400x10^3$/µL). For each patient, there was a sample with a normal platelet count and, at least, a sample with an abnormal platelet count. However, there were some patients with more than one sample with an abnormal platelet count.

Patients with chronic kidney disease, gastrointestinal disease or under treatment with drugs potentially altering potassium homeostasis were excluded (including anti-platelet therapy).

All samples were collected into tubes containing potassium EDTA (whole blood) or serum separator gel (Vacutainer, Becton Dickinson) and received at the laboratory through pneumatic tube. According to laboratory protocols, serum samples were centrifuged at 1500g for 10 min after clot formation.

All biochemical parameters were analyzed within the first hour after blood extraction on an Architect c16000 platform (Abbott Diagnostics). The reference interval for serum K^+ used in our laboratory is 3.6–5.3 mmol/L. Hematological parameters were measured by Hematology Analyzer (Abbott Diagnostics), and the analytical imprecision of the platelet count was 3.02%.

Data analysis

Basal K^+ concentration (A) was defined as the K^+ result with a platelet count within the reference range, while the false K^+ concentration (B) is

referred to the result in the sample with a platelet count above the upper reference limit.

The dependence of K^+ results on the platelet count was assessed by the representation of the percentage variation of K^+ [((B - A)/A) x 100] against the altered platelet result. For individuals with more than 2 samples (more than one with an abnormal platelet count), all differences were referred to the sample with a normal platelet count. All values were included in the analysis.

Statistical analysis

All statistical calculations were performed on the SPSS v.24 software (IBM Corporation, USA). The Kolmogorov-Smirnov test was used to assess distribution normality, and the correlation between variables was evaluated by means of Pearson's correlation coefficient if normally distributed and Spearman´s ρ if not. Statistical significance was set at 0.05.

The cut-off value was determined as the platelet count for which the K^+ result exceeded the RCV of our laboratory. Its related sensitivity and specificity were determined using a receiver operating characteristics (ROC) curve. RCV was calculated using the following equation:

$$RCV = 11.6\% = \pm \sqrt{2} \times Z \times \sqrt{CV_A^2 + CV_I^2}$$

where CVA is the analytical coefficient of variation in our laboratory (most adverse level: 1.3%), CVI is the intraindividual biological variation (4.8%) according to the guidelines of the Spanish Society of Laboratory Medicine (SEQC),[12] and Z is the unidirectional statistical coefficient (1.65 for 95% probability).

A statistically significant result only indicates that it is unlikely that the relationship found between variables is due to chance. However, it does not provide information about the strength of the relationship (size of the effect) or if such relationship is clinically significant. On this basis, the clinical relevance of the thrombocytosis interference was quantified by the effect size estimates using the Cohen's d (parameter generally used to refer to the magnitude of an outcome result or to the strength of the relationship between two variables, in our case, platelet count and K^+ concentration). It was calculated by estimation of the magnitude of the difference between averages of the effect obtained by thrombocytosis (false K^+ results) compared to a control group (basal K^+ results):

$$Cohen's\ d = \frac{|mean_{basal\ K^+} - mean_{altered\ K^+}|}{pooled\ standard\ deviation}$$

Values for Cohen's d less than 0.2 indicate a very low effect, while values greater than 0.8 imply a significant effect.

Clinical consequences

After setting the cut-off value for platelet count for statistically significant interference, false K^+ results corresponding to altered platelet counts were mathematically estimated using the obtained regression, thus obtaining corrected K^+ values. Patient samples with corrected K^+<3.6 mmol/L were classified as PNK episodes, those samples with 3.6≤K^+≤5.3 mmol/L (corrected values within reference interval) were classified as PHK and those with corrected K^+>5.3 mmol/L were classified as true HK.

The clinical scope of this alteration of K^+ results was assessed in two branches.

First, a review of the medical records of the individuals previously identified as cases of PNK, PHK and HK was conducted, aiming to classify clinical outcomes of such actions for the patient. Medical actions aiming to correct potassium were searched, as well as adverse outcomes of over- or undertreatment.

Secondly, a retrospective search was performed for serum K^+ results representing susceptible episodes of PNK or PHK for year 2019. The following filters were applied: K^+ = 3.6–4.7 mmol/L (for PNK) or K^+>5.3 mmol/L (for PHK); platelet count above the established cut-off value; red blood cell count (<5.8x10^6/µL); leukocyte count <20x10^3/uL; serum creatinine <106.1 µmol/L and hemolysis index ≤0.003 g/dL free Hb.

RESULTS

Fifty-four patients with ET met all inclusion criteria for our study, with a total of 94 results. The main characteristics of patients are shown on Table 1. The correlation between the percentage variation of serum K^+ and the platelet count in whole blood was statistically significant (P-value <0.001). These variables were found to be associated in a linear manner, following the equation:

$$\Delta K^+, [\%] = -2.16 + 2.3x10^{-5} \cdot platelet\ count\ [\mu L^{-1}]$$
$$Spearman's\ \rho = 0.394$$

The application of the RCV in the obtained equation yielded a cut-off value for platelet count of 598x10^3/µL [CI 95%: 533–662x10^3/µL] for the definition of interference, with an associated sensitivity of 0.67 [CI 95%: 0.52–0.80] and specificity of 0.58 [CI 95%: 0.42–0.72] (Figure 1). No relationship was found between potassium variation and mean platelet volume (MPV).

The calculation of Cohen's d for the estimation of the size effect of thrombocytosis on the K^+ values yielded d =1.0.

For the correction of serum K^+ results, the established cut-off was used. Of all corrected values, 6.5% (n=7) corresponded to PHK episodes and 1.9% (n=2) to PNK episodes. No results reflecting true HK were found.

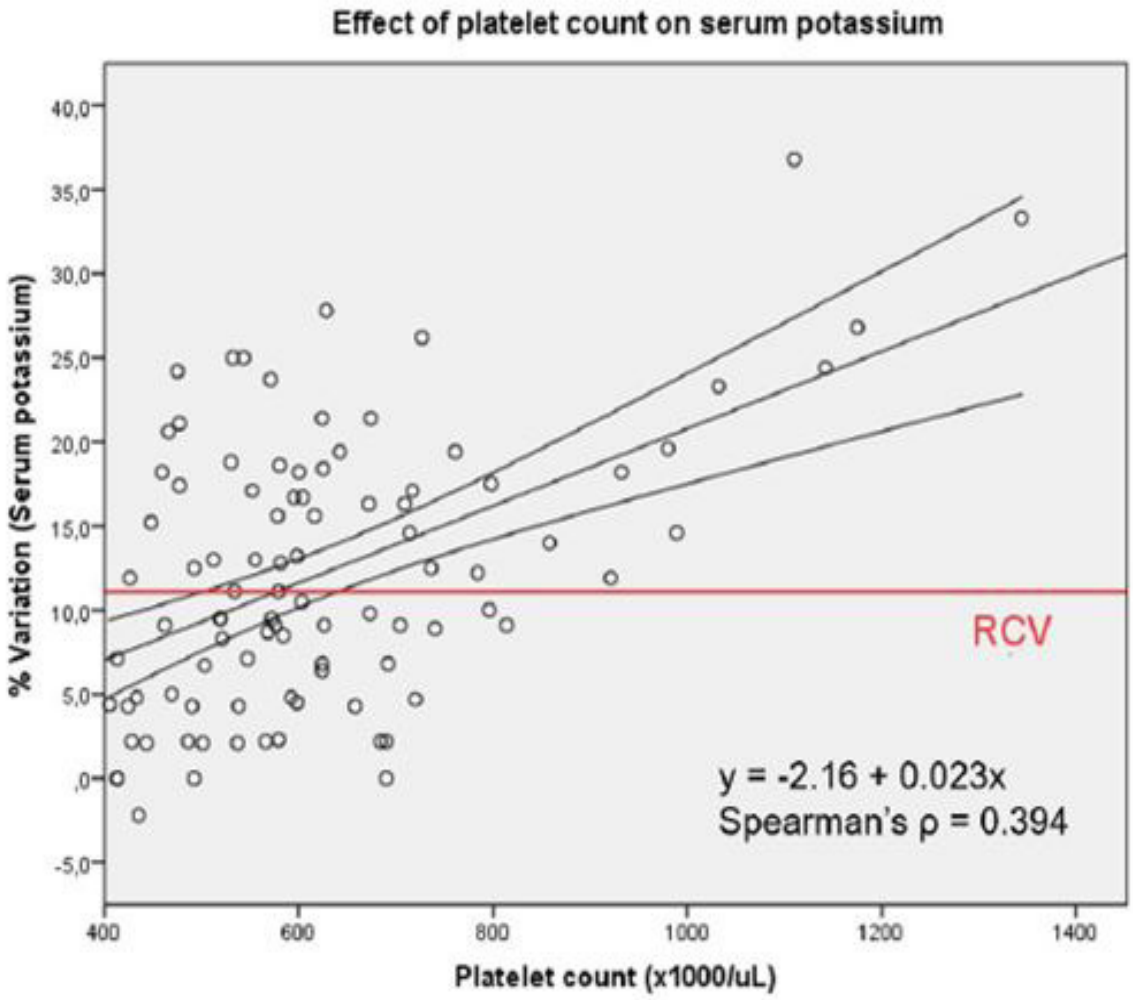
The medical records associated with these 9 episodes did not include any medical action for the modification of K^+ levels. In addition, patients with PNK did not show any adverse clinical evidence for the lack of treatment. Likewise, as a result of the second study, we found out a total of 430 results susceptible of being PNK and 75 of PHK for year 2019 (of over 368,000 K^+ serum tests). This means that 0.14% of the K^+ results may have been incorrectly interpreted in case of thrombocytosis.

Table 1 **Characteristics of patients with ET (samples with an abnormal platelet count)**

	Age	K^+ (before correction), n_R*		Classification (after correction), n_R			Platelet count
		Normokalemia	Hyperkalemia	PNK	PHK	HK	
ET (n=54)	63 [22 – 95]	4.7 [4.5–4.9], 87	5.6 [5.4–5.8], 7	2	7	-	592 [509–719] x10^3/µL

n_R: number of results*
Data median [min-max]. Age
Data median [IQR, interquartile range]. Platelet Count; K+ (mmol /L)

Figure 1 **Impact of platelet count on serum K+ measurements regarding to the baseline values**



DISCUSSION

The retrospective analysis of patients with ET allowed us to verify the dependence of in vitro serum K^+ with platelet count, as previously reported elsewhere.[13]

There is scarce literature regarding the establishment of a cut-off value above which serum K^+ is significantly interfered by thrombocytosis, and the available cutoff values are all based on a fixed change in K^+, independently of its baseline concentration. Thus, Thurlow et al[7] and Ranjitkar et al[14] reported that platelet counts above $500x10^3/\mu L$ cause a variation in K^+ of >0.5 mmol/L. In our study, exclusion of hospitalized individuals was considered, thus assuring that patients with kidney disease, under fluid therapy or under treatment with other drugs potentially altering K^+ homeostasis would not affect the findings.

Moreover, a new feature of our approach was that only effects above the reference range were included in the study (this explains why basal platelet counts were chosen close to the upper limit of reference), leading to a higher robustness of the conclusions. In our opinion, the abovementioned cut-off values could be optimized more accurately since, given the RCV in our laboratory, for individuals with basal K^+ <4.0 mmol/L variations below 0.5 mmol/L could be significant, whereas for individuals with basal K^+ >5.0 mmol/L, variations >0.5 mmol/L would be needed for thrombocytosis to be considered responsible for the increase.

The application of the abovementioned exclusion criteria for individuals diagnosed with ET allowed excluding results potentially generating a bias in the statistical analysis, hence optimizing the selection of data from previous studies.[7,14] In addition, considering the individuality index (II) of serum K^+ (II = 1.0 according to the Biological Variation Database of the European Federation of Clinical Chemistry and Laboratory Medicine, EFLM),[15] the RCV was considered as the statistical element to establish the magnitude of interference.

Regarding the statistical results, the coefficient of correlation obtained for the regression is in accordance with related studies, although the sensitivity associated with the cut-off obtained in our study is lower than that reported by Thurlow et al,[7] probably due to the dispersion of our results.

The clinical relevance of thrombocytosis in the determination of serum K^+ is evidenced by the result of Cohen's d coefficient.-

Pseudohyperkalemia is a finding well known by physicians attending individuals with ET, which is brought to light by the absence of unnecessary clinical interventions in the medical records reviewed in our study. Nevertheless, a professional unfamiliar with this condition may be confused by elevated potassium levels without accompanying reports on hemolysis. This may result in unnecessary treatments leading to potentially dangerous outcomes such as iatrogenic hypokalemia.[16] These observations point out that laboratory reports should include caution notifications if PHK is suspected. Correction of K^+ results in cases of possible PHK could also be a useful alternative to avoid overtreatment, although further studies need to be performed in this direction. Similar strategies have also been suggested for PHK due to hemolysis.[14,17]

Regarding pseudonormokalemia, to the best of our knowledge, this is the first study assessing this phenomenon in clinical samples. Although in our study no cases of moderate to severe hypokalemia were found, the lack of knowledge of this situation and the lack of warnings could increase the risk of underestimation with potential adverse clinical outcomes.

The retrospective study using our cutoff value for cases susceptible of PHK and PNK in our population offers a broad vision of the scope of the interference and the magnitude of potential repercussions being in our case, particularly remarkable the susceptible episodes of PHK. In our study, 8 of the results show moderate (K^+= 6.1–6.5 mmol/L) or severe HK (K^+ >6.5 mmol/L), which means a high probability of carrying out a medical action if the platelet count has not been considered. Therefore, it would be interesting to add such warnings as an aid for the physician towards an improvement in patient safety. Nevertheless, prospective studies comparing serum and plasma samples in patients with thrombocytosis are still needed for the verification and validation of our results.

Translation of research into routine laboratory practice is fundamental. The first steps for the application of our findings should be to include algorithms into the LIS, so that potentially interfered potassium values appear with a comment

and/or those results are held for specialist review. Alternative strategies could be to request the determination of K^+ concentration either in lithium heparin (LH) plasma or in whole blood samples with balanced LH to confirm the presence of the interference.[10]

This study has some limitations, mainly related with its retrospective nature and the trust in the records from the laboratory and hospital information systems. A larger sample size for the establishment of a cut-off value for the platelet count would add robustness to our conclusions, as the dispersion of our results leads to a low coefficient of correlation. This could also be partially explained by the interindividual biological variability, which was not included as variable in this study. Besides, result correction should always be performed with care and potential misclassifications should be studied. The decision on using plasma samples instead of serum in the measurement of potassium lays in each laboratory, depending on their organization and the distance of blood collection points. In addition, other unusual potential sources of contamination, such as EDTA contamination, were not specifically tested.

As strengths, this study provides an improved cut-off value by specifically selecting the participants: platelet counts homogeneously distributed in the pathological region above the upper limit of reference and avoiding hemolyzed samples. In addition, this study brings to light not only PHK-related issues and its overtreatment consequences, but also the PNK phenomenon, which may usually go unnoticed and whose lack of treatment might carry adverse outcomes for the patient. Thus, as individuals with platelet counts above the cutoff value could present potassium values exceeding the RCV, those results should be interpreted cautiously. Although the authors are aware that our cutoff value is not optimal, they consider it is of a great clinical usefulness, given that a platelet count above such cutoff value leads to a significant (false) increase in potassium values, with subsequent potentially adverse medical actions. Future studies warrant a better adjustment and optimization of the regression and cut-off values.

In conclusion, and considering hypo- and HK as life-threatening disorders, with an essential early detection and treatment, laboratory professionals need to identify possible interferents and remove or minimize them, so that the K^+ result on the laboratory report is accurate. This will allow physicians to take medical actions according to the real needs of the patient and avoid under- or overtreatments, thus enhancing patient safety.

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Declaration of competing interests

All authors declare no conflicts of interest.

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Ethics approval and consent to participate

The study was approved by the Ethics Board of our institution [Research Ethics Committee of the Balearic Islands (CEI-IB), nº IB 4191/20 PI].

Contributorship

Study conception and design: JAD, DMG, BL, JMB; acquisition of data: JAD, MAB, SAJ; analysis and interpretation of data: JAD, JMB; drafting of manuscript: JAD, JMB, BL, EMM. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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Urinary screening in asymptomatic Indian children: a cross sectional epidemiological study

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chronic kidney disease, hematuria, pediatric population, proteinuria, urinary screening

ABSTRACT

Background and aims

Early detection and management of renal abnormalities in children can reduce the progression of paediatric chronic kidney disease. Currently, data on the prevalence of routine abnormal urinary parameters are scarce in Indian population. This study aims to identify the prevalence of asymptomatic kidney diseases in Indian school children and the population who may benefit from routine urinary screening tests for timely identification and intervention of asymptomatic renal diseases.

Materials and methods

A total of 1675 children from a North Indian, multiethnic population aged 5-19 years were screened for hematuria and proteinuria by dipstick test from a midstream, clean urine specimen. The children who tested positive had their urine tested further for

microscopy. The incidences of proteinuria and hematuria were also separately checked in hypertensive children.

Results

76 children had urinary abnormalities with the prevalence of isolated haematuria in 1.9%, isolated proteinuria in 0.35% and glycosuria in 0.06%. When these children were followed with urine microscopy, 44 were observed to have abnormal findings. Of these, 4.5% children had proteinuria, 34% had isolated hematuria, and 47.7% had isolated WBCs. The prevalence for proteinuria was 0.60% and the prevalence for hematuria was 2.99% (in upper decile of SBP) in hypertensive children, both of which were more than the prevalence in otherwise healthy children.

Conclusion

Urine screening is a non-invasive, inexpensive test for early detection of occult renal diseases. A large-scale study with follow-up of children with urinary abnormalities will further establish the benefit, if any, of a national paediatric urine screening programme.

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1. INTRODUCTION

Chronic Kidney diseases (CKD) in children, although relatively uncommon, can be a devastating illness with long-term consequences. Data on prevalence of routine urinary abnormalities are unfortunately very scarce in Indian population when compared to global data. Early detection and management of kidney disease would reduce the progression and therefore national burden of paediatric CKD. Paediatric societies and government regulation in certain countries suggest routine periodic urine analysis in children for early diagnosis and timely management of CKD in children. Presently there is no national epidemiologic data on prevalence of CKD among Indian children and therefore the proportion of children who would possibly benefit from a routine periodic urinary screening tests are uncertain.

Urinalysis is a simple and inexpensive test which is the cornerstone in the evaluation of kidney functions. It can be easily employed in screening of renal abnormalities. Several urinary screening programs have been carried out using reagent strips, and their effectiveness in detecting urinary abnormalities at relatively low cost has been evaluated [1,2]. Abnormalities detected in routine urinalysis in patients who have no symptoms of renal or urologic disease such as glycosuria, pyuria, haematuria, and proteinuria are a common finding in clinical practice. Renal diseases are often accidentally discovered during routine urine analysis in asymptomatic healthy individuals [3]. With the aid of routine dipstick examinations early symptoms of diseases of the kidneys and the urinary tract (pyuria, haematuria and proteinuria) can be identified. An abnormal urinary test may be the earliest warning of a significant renal pathology [4,5]. Mass urinary screening helps to determine the prevalence of renal diseases and to improve the outcome in the population [6,7].

Although the incidence of urinary abnormality may be clinically insignificant or false positive in certain cases, studies from Korean, Taiwanese and Japanese paediatric screening program indicate a clear advantage of early detection and effective intervention to prevent progression to End-stage Renal Disease (ESRD) [8]. Murakami *et al.* carried out a large-scale screening among Japanese children and reported a fourfold decrease in incidence of progression to ESRD among Japanese children when compared to the US. Although the lower incidence of ESRD among Japanese children might be multi-factorial, early screening and timely management is a crucial factor.

Common causes for renal abnormalities in children include haematuria due to renal stones, structural deformities, urinary tract infection, glomerulonephritis, or proteinuria due to nephrotic syndrome can be effectively screened by routine urine analysis. Therefore, the present study was planned to determine the prevalence of occult renal diseases in asymptomatic school children & adolescents. The findings of the study may aid in identification of prevalence of asymptomatic renal diseases in Indian children and proportion of children who may benefit from routine urinary screening tests resulting in timely identification and intervention of asymptomatic renal diseases.

2. METHODOLOGY

2.1 Ethics

The study was carried out following the principles of the Declaration of Helsinki regarding medical research involving human subjects. Ethical clearance was obtained from the Institutional Ethics Committee. Informed consent was obtained before enrolling all participants in the study.

2.2 Study population and screening protocol

The present study is a cross sectional epidemiological study carried out in India. The study subjects were apparently healthy children attending a leading school in Jammu and Kashmir, India. The study was carried out among asymptomatic school children of 5-19 years of age within the span of one year. The children came from multiethnic background from all socioeconomic strata of Northern India. Sampling was carried out following a simple random sampling method. Children with pre-existing renal or any other systemic diseases, children on steroid therapy, and children whose parents refused to give consent were excluded. A total of 1675 students were recruited after obtaining informed consent from parents. Participants were instructed to void a mid-stream clean urine specimen into a 100 ml vessel, which was examined by a trained lab technician with the help of analyser.

The study was started in a small lab established at the school for the period of two months. The first morning urine sample was obtained from each child in a clean 100 mL vessel, which was tested with a urinary dipstick (Multistix, Nicolas Piramel) for haematuria and/or proteinuria as a first screening test. The second screening test was performed 2–4 weeks later by microscopic method on 44 children who had tested positive in the first screening. Blood investigations, including renal function, liver function and lipid profile, as well as abdomen ultrasonography (USG) were carried out.

2.3 Dipstick analysis

Dipstick test (Multistix, Nicolas Piramel) was performed on the unspun urine specimen with reagent strip designed to react progressively producing color changes at given intervals. The results were decided by visual comparison of the test strip with a color chart provided on the bottle label.

Urinalysis was considered abnormal by dipstick if the following findings were detected:

1. Haematuria if >5 RBC/µl (Green dots on yellow test: intact erythrocytes; Uniform green coloration of test: free hemoglobin or hemolysed erythrocytes);

2. Proteinuria (>30 mg/dl)

3. Glycosuria (>100 mg/dl)

4. Leukocyturia (>25 WBC/µl)

Haematuria

According to the American Urological Association, the presence **of three or more** red blood cells (RBCs) per high-powered field (HPF) in two of three urine samples is the generally accepted definition of haematuria [9,10].

Proteinuria

It is defined as urinary protein excretion of 30-150 mg/day and is the hallmark of renal disease. As per reagent kit insert, clinical proteinuria is defined with strip result of > 30mg/dL. Microalbuminuria is defined as the excretion of 30-300 mg/day of protein and is a sign of early renal disease.

Glycosuria

The urine analysis has been performed using Multistix reagent strips. Small amounts of glucose (<30 mg/dL) was below the sensitivity level of this test. The sensitivity of the test was 75-125 mg/dL and a value above 100 mg/dL was interpreted as positive result by the Multistix reagent strips

Pyuria

It is defined as ≥6 WBC/HPF in the urine sample by microscopic method.

2.4 Hypertensive population

The hypertensive population was determined by sorting the children having systolic blood pressure (SBP) or diastolic blood pressure (DBP) above the 90th percentile. The selected individuals were then checked for the presence of proteinuria and hematuria.

2.5 Statistical analysis

Data were analysed using Microsoft Excel and RStudio. Qualitative data were expressed in the form of numbers and percentages. Comparison between data was performed by using the Chi-square test. P value <0.05 was considered statistically significant.

3. RESULTS

3.1 Prevalence of urinary abnormalities

The study sample consisted of 1675 children with a male to female ratio of 1.53:1. In total, 76 children had urinary abnormalities with the prevalence of isolated haematuria in 1.9%, isolated proteinuria in 0.35% and glycosuria in 0.06% (Table 1). Under urine microscopic examination, urinary crystals were observed in 9 children. When 76 children with abnormal urine dipstick tests were followed with urine microscopic examination, 44 children were observed to have abnormal findings (Figure 1). Of these 44 children, 4.5% children were having protein in urine, 34% had isolated RBCs, and 47.7% were found to have isolated WBCs in urine (Figure 2).

Two children had both hematuria and urinary crystals on follow-up, while one other child simultaneously had pyuria and the presence of urine crystals. Interestingly, none of the children

Table 1 Descriptive statistics of children having urine abnormalities by dipstick method (N=76)

Abnormality	N	%
Proteinuria	4	5.2
Haematuria	32	42.1
Glycosuria	1	1.31
WBC	39	51.3

Figure 1 Number of pediatric population at each step of the screening

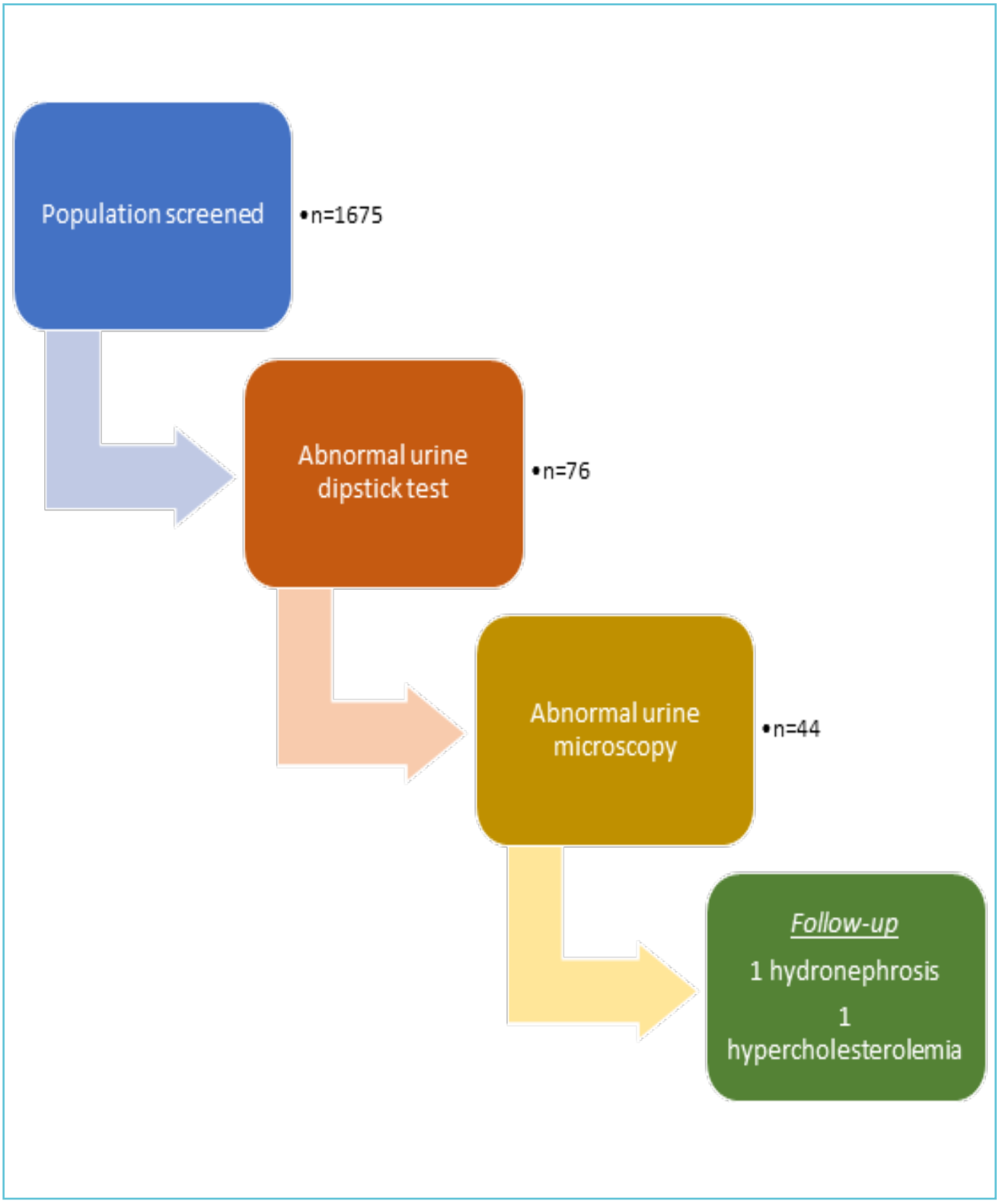
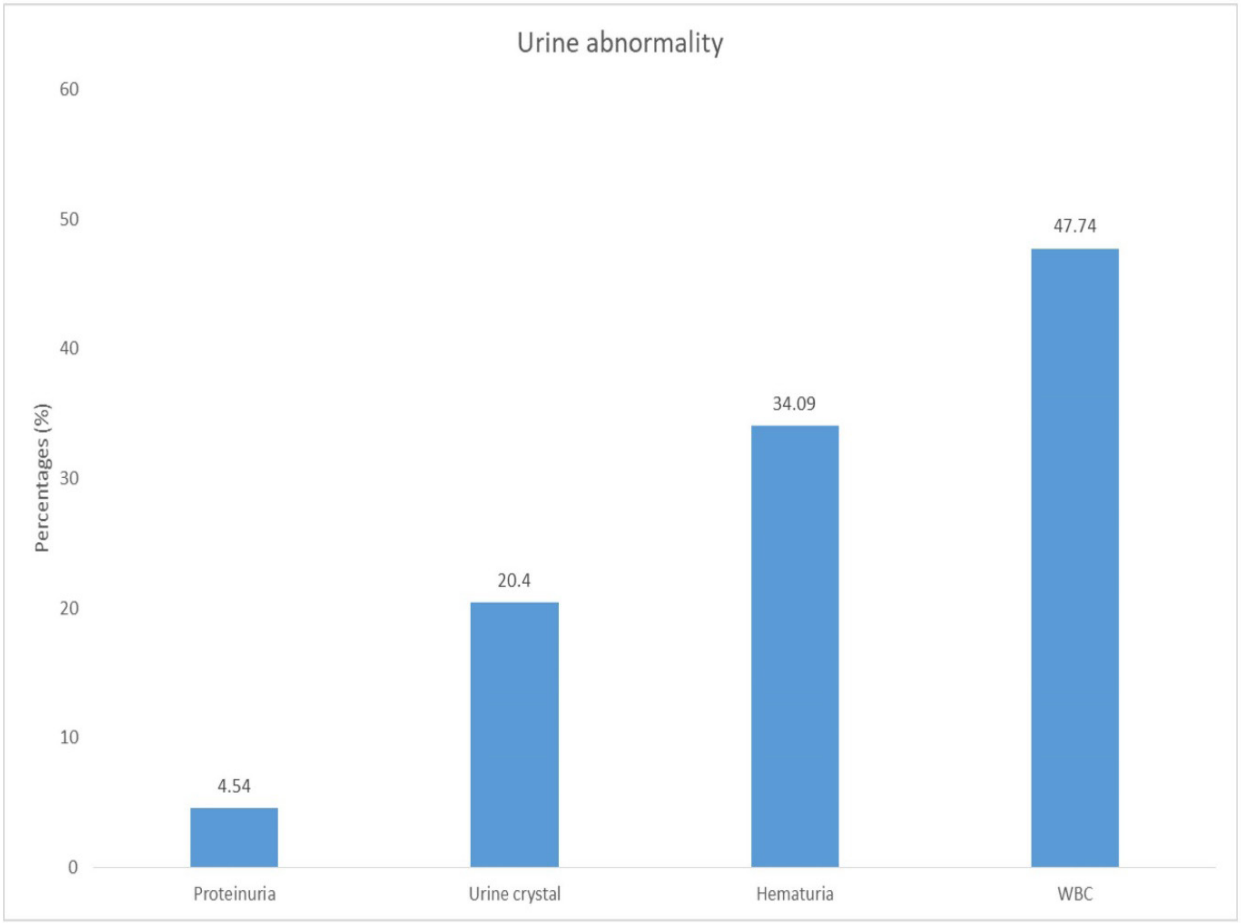


Figure 2 Prevalence of urinary abnormalities as per urine microscopy (n=44)



20 children had pyuria, 13 had hematuria, 6 had urine crystals, and 2 had proteinuria. 2 children had both hematuria and urinary crystals, while 1 child simultaneously had pyuria and the presence of urine crystals.

Table 2 Proteinuria and hematuria in hypertensive children

	SBP >90th percentile N (%)	DBP >90th percentile N (%)
Proteinuria	1 (0.60)	1 (0.60)
RBC+	5 (2.99)	8 (4.79)

Hypertensive children were sorted according to SBP or DBP above the 90th percentile cut-off.

For SBP, there was one case of proteinuria and five cases of hematuria above the cut-off, whereas for DBP, there was one case of proteinuria and 8 cases of hematuria.

had the presence of hematuria and proteinuria together. Further, when these 44 children were followed up with USG and blood investigations (renal function, liver function, and lipid profile), one child was identified to have hydronephrosis (right kidney) on USG, and another had hypercholesterolemia.

3.2 Blood pressure and urinary abnormalities

A high SBP of >130 mmHg was observed in 15 children and DBP >80 mmHg was observed in 19 children.

There was 1 case of proteinuria and 5 and 8 cases of hematuria, respectively, in the upper deciles of SBP and DBP. The prevalence for proteinuria was 0.60% and the prevalence for hematuria was 2.99% (in upper decile of SBP) in hypertensive children, both of which were more than the prevalence in otherwise healthy children (Table 2). Overall, the study found a prevalence of 0.12% proteinuria, 1.91% haematuria and 1.25% pyuria in otherwise healthy children. Those with abnormalities were referred to pediatrician/pediatric nephrologist for detailed evaluation.

4. DISCUSSION

CKD in children, although uncommon in nature, can cause devastating illness with long-term consequences in children. The current increase in the incidence of paediatric obesity leading to increased incidence of hypertension, may further contribute to increased burden from renal disorders in children [11]. Indeed, in the 44 children with renal abnormalities in follow-up, two children were obese; and both of them had SBP and DBP above the 90th decile. The prevalence of proteinuria and haematuria in hypertensive children which is more than normal underscores the importance of long term follow up in these children so that progression to Chronic Kidney disease can be monitored. Renal diseases in children can be silent (asymptomatic) in early stages and advance to ESRD requiring dialysis or renal transplantation. Mortality of children undergoing dialysis for ESRD is much greater (30-100 times) than general paediatric population [12].

Routine urine analysis is a simple and effective means to screen for potential underlying renal disorders. Routine mass screening in paediatric population has been a part of national scheme in countries like Korea, Taiwan and Japan. Routine urine screening programs are recommended as a basic fundamental step in early identification of renal damage. This has proved to be extremely important in reducing the growing burden of CKD in both developed and developing countries. In the present study, urinary abnormalities was present in 2.6% of the studied group which was comparable to that reported in Northern Iran (2.5%), Malaysia (2.3%), Tokyo (0.6%) and Egypt (0.72%) and lower than the 7.2% and the 9.6% reported in Bolivian and Nigerian studies respectively [13–17]. A cross-sectional study on 1597 Indian children aged 5-16 years revealed a prevalence of urinary abnormalities at 7.82% [18]. Proteinuria, haematuria, and pyuria were found in 4.3%, 5.2%, and 2.5% of school children aged 6-18 years in a study conducted by Vinoth et al. [19]. They also found that the urinary abnormalities were more prevalent in males.

Haematuria was the most common abnormality found in our study group in agreement with Vinoth et al [19]. This was in contrast to other studies in Egypt and Nigeria where proteinuria was the most common positive finding [16]. Hematuria had a prevalence in Malaysia, Egypt and Shanghai (0.21%, 0.36% and 0.46% respectively) which is comparable to our results which showed a prevalence of 1.91%. Nigeria and Xiamen City (China) reported a comparable prevalence of 1.5% and 1.21% respectively [15,16,20]. In an Indian study, hematuria was found in 5.8% children [18]. Infection constitutes of 14% of gross hematuria in children, according to one study [21]. Among these, parasitic hematuria,

particularly due to Schistosomiasis, is prevalent in tropical and subtropical countries [22]. A careful history and physical examination as well as focused laboratory investigation may provide sufficient insight in these cases. Bergstein et al. evaluated 342 children referred to their nephrology clinic for asymptomatic isolated microscopic haematuria. Among these patients, they found no abnormality in 274 children [23]. Other authors like Vehaskari et al. performed biopsy from 22 children with microscopic haematuria having no family history of kidney disease and a negative evaluation for causation. All but three biopsies were normal and showed non-specific focal tubular changes [24]. In a screening study done in Japan, they found 6 cases of IgA nephropathy and 7 cases of minor glomerular abnormalities among 220 children with asymptomatic haematuria [15]. These studies suggest a benign nature of microscopic asymptomatic haematuria that may be an important sign of underlying disease. However, limitations of these studies were the absence of long term follow-up and thus, the frequency of development of complications and occult kidney disease was not known.

Furthermore, in patients with microscopic haematuria from occult glomerular disorders, progression to clinically significant disease will be accompanied by the development of hypertension with or without proteinuria or gross haematuria. Thus, long term follow-up in children with microscopic haematuria is crucial. Proteinuria can be a major cause of underlying kidney disease or a transient finding in normal children. In our study, first morning urine sample helped in excluding orthostatic proteinuria as a cause of isolated proteinuria in children. The dipstick is mainly sensitive to albumin, whereas quantitative methods detect all kidney proteins. Proteinuria is a strong and independent risk factor of ESRD. Therefore, asymptomatic proteinuria warrants further work up to detect and even prevent ESRD [25]. Furthermore, an increased prevalence of proteinuria in hypertensive children demands more attention towards renal work-up to prevent the possibility of renal diseases in the future.

Until recently, American Academy of Paediatrics (AAP) had recommended routine urinalysis by dipstick method for children under 5 years of age. However, with the recent evidence of low incidence of CKD from multiple large-scale studies in paediatric population, AAP no longer recommends routine urinalysis in children. Prevalence of paediatric CKD, cost of screening and burden of ESRD are the important factors in determining the effectiveness of routine urinary screening programmes. Studies from Korean, Taiwanese and Japanese paediatric screening program indicate a clear advantage of early detection and effective intervention to prevent progression to ESRD [8]. All these studies emphasize the importance of following up children with silent renal diseases.

5. CONCLUSION

Our study found a prevalence of 0.12% proteinuria, 1.91% haematuria and 1.25% pyuria in otherwise healthy children in a North Indian population. Those in whom these abnormalities were found were referred to pediatrician/pediatric nephrologist for detailed evaluation. Urine screening is a feasible, non-invasive, and inexpensive test for early detection of occult renal diseases, which can be helpful if incorporated into school health programmes. A structural framework can also be developed based on data for the diagnosis, prevention and management of renal diseases. Due to scarcity of national data on prevalence of renal abnormalities in Indian children, the national burden of providing medical care for children with CKD remains unknown. A large-scale study with follow-up of children with urinary abnormalities will further add to the findings of our study and establish

the benefit, if any, of a national paediatric urine screening programme.

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Author contributions

Mithu Banerjee: Conceptualization, Methodology, Investigation, Writing-Reviewing and Editing.

Dipayan Roy: Software, Writing-Reviewing and Editing, Visualization.

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Assessing third-year medical students' perspective on point of care testing boot camp: from bench to bedside

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ABSTRACT

Background

Point-of-care testing (POCT), which is also known as bed side-testing, has been integrated into the healthcare system, offering faster results that can lead to improved patient outcomes. POCT was missing from the medical education curriculum in our institute.

Objectives

The primary objective of this study was to describe the development and introduce POCT training for medical students in a medical college in Pakistan.

Secondary objectives were to evaluate student performance on POCT content and to assess the impact of POCT training via students' feedback.

Methodology

The boot camp experience was devised, directed, and facilitated by team constituting of Chemical Pathology faculty members, laboratory technologists and teaching assistants. The program included presentations, demonstrations of POCT instrument handling, supervised hands-on individual performance on glucometer using quality control specimens, competency assessment and sign off followed by interactive case-based discussions. A knowledge quiz via Kahoot was administered at the beginning and end of the experience and scores were compared statistically. Online evaluation and feedback were designed via virtual learning environment based on 10 questions regarding the program and methodology using on a five-point Likert Scale. Frequencies were generated and t-tests were employed to determine pre-post differences.

Results

The boot camp was spread over 2 days and ran three hours each day with the third-year medical students class split into two groups (n=80). On knowledge evaluation, the mean group pre and post test scores were 45% and 95% respectively (p-value =< 0.05). On documented structured competency assessment form a score of 95 was achieved by 100% participants. Positive feedback of 4 or more was recorded on the Likert's scale by 100% respondents.

Conclusion

This POCT boot camp experience can be used by other institutions and can be applied at different times during the medical school curriculum and other professional education programs. This bootcamp will be helpful to educate medical students, postgraduate trainees and field workers working in rural areas and in low resource settings to deliver reliable POC tests results. Future research should examine these students' competence in achieving POCT skills when they enter in clinical practice.

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INTRODUCTION

The POCT working group of the International Federation of Clinical Chemistry (IFCC) defines Point-of-care testing (POCT), *'as diagnostic testing undertaken at or near the site of the patient'* (1). Similarly, The College of American Pathologists (CAP) terms POCT as *'testing that does not require permanent dedicated space and it refers to those analytical patient-testing activities provided within the institution but performed outside the physical facilities of the clinical laboratories'* (2). Majority of the medical decisions are made on laboratory investigations (2). POCT can be advantageous in clinical situations requiring rapid turnaround time of test results for clinical decision making. Medical students or the future doctors should understand POCT and its clinical utility as they will eventually be the end users of POCT and hence need knowledge of the issues surrounding POCT compared to testing in a hospital laboratory and should ensure tests performed outside laboratories meet appropriate quality standard. However, there is a gap in pathology teaching related to POCT between what the medical graduates are competent of and what these graduates are required to learn (3). Exit competencies related to POCT in medical education do not exist, neither there is any standardized curriculum for POCT to teach principles of best practice in POCT in medical schools, schools of nursing, pharmacy and medical laboratory technology science.

In medical schools in Pakistan and globally, since long pathology was taught traditionally as a standalone 6- to 12-month course with focus on pathogenesis more from the anatomic pathology perspective, with little emphasis on laboratory

medicine (4). Over the past few decades many medical schools have modified their curricula, in a more-integrated context. As a result, medical students are presented with broader learning concept from bench to bedside (5). Even though laboratory medicine teaching has been on the horizon, POCT remains a neglected area (6). An early practice-based understanding of POCT is essential for medical students before transitioning into patient care (7).

Generally, currently medical curricula are intended to assist medical students to link clinical and basic science knowledge. Taking example of glucometer as a POCT device and diabetes where prior knowledge of basic sciences (like pathophysiology of diabetes) can be integrated with clinical application (like the need of testing, interpretation of glucose levels, importance of accuracy of results) from the early years of medical training. The overall aim of the current study was to develop an integrated POCT curriculum incorporating clinical and basic sciences concepts, choose relevant patient cases and guide student discussions (8).

The primary objective of this manuscript was to describe the development and implementation of POCT training for third-year medical students at the Aga Khan University Medical College. Secondary objectives were to evaluate student performance on POCT content and to assess the impact of POCT training through students' feedback.

MATERIAL & METHODS

This study was conducted at the Aga Khan University Medical College, Pakistan in the year 2021 after approval from ethical review committee of the Aga Khan University (2021-6938-19677). This was conducted in four phases as described below. A hands-on interactive POCT boot camp was developed and conducted in year three of the medical curriculum.

Phase I – team formulation and course content design

The plan was devised and directed by a team lead, and three other chemical pathology faculty members and two laboratory scientists (working as POCT coordinators) who constituted the POCT-medical education team. The team was formulated as such to include a blend of practical, theoretical and clinical skills side by side. Each case scenario was further developed by a faculty with special interest or expertise in the specialty topic. Similarly, the POCT coordinators based on their expertise in procedural skills provided hands on training and conducted the competency assessment.

To build on ideas for the content design and delivery, in the first phase brain-writing process was carried out in multiple small group discussions. After thorough literature review the team lead developed the curriculum and laid down the course content. Due to the Covid pandemic, the discussions were mostly virtual and through emails. Team members devised the objectives of the POCT boot camp centred across a vision to incorporate adequate comprehension of the needs and utility of POCT. The aim was to enable medical students to learn clinical observation, examination and reasoning skills taking POCT for diabetes as an example. Through this activity students will learn to integrate basic science concepts with their clinical experience to nurture their diagnostic reasoning skills (9, 10). Clinical cases with POCT data were added in the boot camp so that the students can make sense of the clinical information they gather. A plan was laid down to instil interactive discussions on clinical cases and make them relate potential pathophysiological mechanisms with the clinical and POCT data findings (11).

The team decided to use the Sawyer et al. simulation-based framework for POCT devices and procedural skill training which includes six

steps: learn, see, practice, prove, do and maintain (12). In this framework, participants will be offered deliberate practice and instructor feedback to achieve procedural competency. In line with the theory, the team developed content to support each outlined step.

Phase II – course delivery

The POCT training was implemented in a required clinical skills course for third year medical students. Based on our previous experience of regular training of health care professionals in the POCT program, the intensive and condensed course was planned to be conducted as a boot camp, spread over 2 days and lasted three hours each day with the third-year medical students' class split into two groups (2). The team members trained five teaching assistants to facilitate the boot camp along with them and were instructed to act as master trainers and assessors for the boot camp. The teaching assistants at our institute are fresh graduates of the medical school serving as aid for faculty. The pre training of teaching assistants was conducted on the glucometers using quality control specimens and competencies were documented.

Phase III – evaluation & feedback

A key component was simulation-based mastery learning i.e., learners must prove their procedural competency by passing a summative assessment (13). To aid this a predefined competency assessment checklist was formulated. A knowledge quiz via *'Kahoot!'* was developed for administration at the beginning and end of the experience (14). *Kahoot!* is a free cloud based digital learning platform that uses quiz-style games to help students learn by making the information engaging in a competitive way. Using Kirkpatrick's suggested framework for evaluating the results of training based on four important levels of training evaluation: reaction, learning, behavior and results, a feedback questionnaire was designed. Online evaluation and feedback were designed via virtual learning environment using Moodle, a virtual course management system provided by the university based on 10 questions regarding the program and methodology using on a five-point Likert Scale. This scale is a type of psychometric response scale in which responders specify their level of agreement to a statement typically in five points (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree and 5 = strongly agree) (15).

Data collection, analysis, and ethical approval

The data entry and statistical analysis was performed using the Microsoft Excel 2013 and Statistical Package of Social Sciences (SPSS) version 19. Descriptive results based on the responses were also recorded. A chi-square test of independence was performed to examine the relation between pre- and post-test scores. Two-tailed p-values < 0.05 were considered significant.

RESULTS

Based on the intense and vivid brain writing session, the ideas generated for imparting the addition in curriculum were further weighted upon in the group meeting, before implementation. The content design was laid in perspective of the Sawyer et al. simulation-based framework for procedural skill training as outlined in table 1 (12). The content was reviewed and approved by the third-year curriculum review committee of the Aga Khan University Medical College.

POCT boot camp was conducted on April 7 & 8, 2022 for 3rd year medical graduates in two groups of 80 students each day. The learning objectives and the program of the session are enlisted in Tables 2 and 3.

On knowledge evaluation, the mean group pre- and post-test scores were 45% and 95%, respectively (p-value= <0.05).

Table 1 Content design and delivery strategy

Teaching element	Teaching strategy
Learn	Presentation on utility of POCT, in-depth overview of POCT operations with key quality control measures. Presentation on diagnostic criteria of diabetes mellitus, critical results reporting and sentinel events avoidance.
See	4.5 minutes video clip detailing step by step procedure of POC test performance using glucometer. Demonstration of use of glucometer by POC Coordinator of the clinical laboratory.
Practice	Supervised hands-on individual performance on glucometer using quality control specimens.
Prove	Competency assessment via check list enlisting all essential steps including preanalytical, analytical and postanalytical. Pre- and post-test via Kahoot.
Do	Each student performed glucose or QC analysis and recorded their results.
Maintain	Skills imparted will be beneficial in clinical rotations in the clinical years and in clinical practice.

On documented structured competency assessment form filled by the facilitator supervising, a score of 95 was achieved by 100% of the participants. Feedback of 4 or more was recorded on the Likert's scale based on 10 questions by 100% of the respondents. Twenty students provided additional comments.

In a nutshell, the comments were positive; centered across inclusion of more similar activities in medical curriculum, hands-on experience was beneficial and interesting, competency assessment documentation was useful, and the cases were full of learning.

DISCUSSION

The COVID-19 pandemic highlighted the importance of laboratory medicine at the forefront (16). POCT is another arm of the subject that rose on the cutting edge (17). However, the foreseeable challenge is the capacity building of health care professionals who can deliver POCT services (18). Medical students if trained in this arena during the early phases of professional education can lead to development of public health advocates who can be utilized for the "train the trainers" modality and also for quality practice in physician office (19).

Figure 1 Content proposal based on deliberated discussions amongst team members

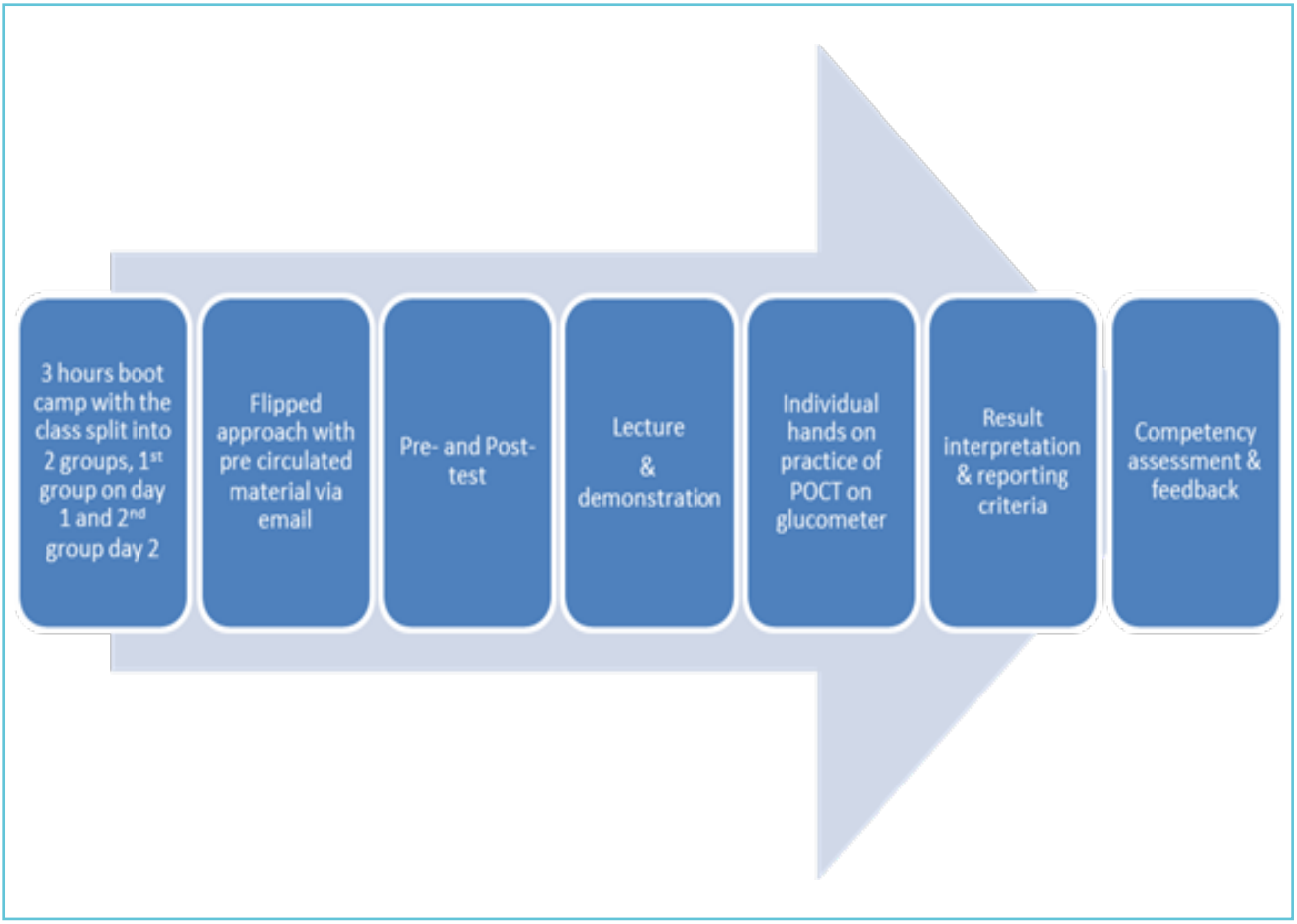

Table 2 Learning objectives of the POCT boot camp

Knowledge	Skills
To understand the functioning, organization and set up of POCT program	To perform glucose testing on glucometer (skill)
To comprehend pre-requisites specimen collection/preservation	To learn effective communication skills for critical laboratory results information and documentation
To interpret the glucose results according to American Diabetes Association guidelines	

To understand the importance of quality control in interpreting glucose results	
To relate the utility of POCT to patient management in Emergency Department and critical care areas	
To understand what sentinel events are and how can they be prevented while conducting POCT	
To understand the importance of correct identification and barcode system to prevent preanalytical error	

Table 3 Course program layout and contact time for each component

Component	Time allocated (minutes)
1. Knowledge assessment via pretest	20
2. Lecture I: POCT utility, implementation, analyzers and techniques available	15
3. Lecture II: POCT quality control, accreditation and key performance indicators	15
4. Demonstration via video and practical performance	10
5. Hands on individual performance	60
6. Competency assessment and sign off	20
7. Case based discussion in four groups emphasizing upon critical results alerts and avoidance of sentinel events	20
8. Post Test-Quick Assessment of data interpretation skills based on diagnostic case challenges of diabetes mellitus	20

Finding the time to teach laboratory medicine principles in an already packed medical school curriculum has proven challenging (20). Keeping this in perspective, the faculty through a force field analysis speculated that the introduction of POCT in medical curriculum will be beneficial which led to the addition of this boot camp to the condensed third year medical students' class at Aga Khan University Medical College in Pakistan.

Pathology and laboratory medicine is not amongst the core clinical rotations at most medical institutes globally (21). Moreover, hands on training in the form of laboratory rotations which can bridge the gap between theory and practice is not routinely included. Eventually most schools do not have any required clinical experiences in pathology for their graduate. This 3-hours long module, with intensive hands-on experience has shown to increase medical students' comfort in performing POCT. All students achieved excellent competency on the pre-designed practice check list on the first attempt. Developing future doctors who are competent and knowledgeable with POCT may lead to increased opportunities to gain real-world experience performing these skills at bedside, and specifically field testing in unanticipated times if required, ensuring optimal quality checks. The POCT boot camp also highlighted the integral role of laboratory medicine in patient management and provided opportunity for questioning and interaction with laboratory personnel and faculty. Besides POCT application and interpretation, the module created understanding for the use, interpretation and limitations of laboratory tests for various clinical purposes like screening, risk assessment, establishing diagnosis, prognosis, etc. It also emphasized on how to order tests necessary and medically useful for patients and seeking consultation from pathologists to maximize patient care (22).

The significant difference noted on post test results speaks for itself, as regards to knowledge imparted within a short span. However, the assessment was able to quantify a limited amount of knowledge attained recently, nevertheless, it was encouraging that improvement could be observed by devoting a short period to learning POCT principles. The test was not mandatory and was not intended to pass or fail a student, neither was it counted as an incentive for completing the module, which may perhaps account for lack of motivation to score well.

Through continuous discussions and deliberations over email and small group meetings, we ensured close contact between team members despite COVID pandemia in a concentrated period. All the cases developed were tailored to the glucose evaluation at POCT to keep the clinical interest of the medical students alive, and the laboratory medicine core foundations were presented alongside. Moreover, the faculty team has planned to review the knowledge examination content annually to ensure the course structure is allowing adequate opportunity for delivery of information.

In addition to its merit of being a first of its kind plan for POCT in medical curriculum, there are limitations to this initiative. This project involved only one program at one university. Future research should involve incorporating POCT training into additional medicine programs to provide comparative data for broadscale evaluation. Also, a follow-up study of these students using satisfaction survey methodology should be planned to assess their ease with POCT usage when they enter clinical practice. There is a growing consensus in medical education about the value of formal competencies for medical students and this program provided structural basis for documented competency assessment of POCT based on the describe measurable and observable behaviors.

CONCLUSION

To conclude this boot camp, our experience can be used by other institutions and can be applied at different stages during the medical school curriculum and other professional education programs including nursing, pharmacy and medical technology. This POCT Boot camp is now an annual activity at Aga Khan University Medical College for third-year students and is part of the new module (Back to Basics) offered during the initial eight weeks of Year 3, MBBS Program. This competency-based program will allow for easy adoption of POCT into existing clinical courses and rotations. Widespread use of sub-standard POC tests is common in resource limited setups with no formal accreditation and quality oversight. This boot camp model has the potential for replication in undergraduation and post-graduation medical education, including nursing, pharmacy and medical technology. as well as training of the field force for POCT community-based projects.

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Data availability statement

The data that support the findings of this study are available from the author on request

Ethics statements

The study was approved by the ethical review committee of the Aga Khan University (2021-6938-19677).

Author Contribution

SA performed the literature search, data analysis and write-up of the work in the first draft. HM, AHK were faculty member, part of the core project team and assisted with manuscript writing, literature search and critical review of the final draft. SS and SJS as POCT coordinators who provided technical support and training. LJ conceived the idea, supervised the project, coordinated the writing of the paper and reviewed the final draft. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Nephrotic syndrome and Hodgkins lymphoma – an unusual association

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Key words:
proteinuria, paraneoplastic syndrome, Reed Sternberg cells

ABSTRACT

Background

An association between nephrotic syndrome and extrarenal neoplasia was described for the first time in 1922. The reported incidence of nephrotic syndrome in Hodgkin lymphoma is less than 1%.

Clinical description

We present a 13 year old boy who was admitted with complaints of abdominal pain, vomiting and loose stools for 2 months. He had a history of significant weight loss of 5kg in a couple of months.

On examination, he had bilateral pedal oedema and right cervical lymphadenopathy. Cervical lymph node biopsy revealed nodular sclerosis type of Hodgkin lymphoma. He also had hypoalbuminemia, massive proteinuria and hypercholesterolemia.

Secondary nephrotic syndrome due to Hodgkin's lymphoma was made as a clinical diagnosis.

Management and outcome

He had been started on chemotherapy (with Prednisolone, Vincristine, Doxorubicin, Etoposide) for stage 3B Hodgkin lymphoma. He tolerated the chemotherapy well. Though he had symptomatic edema, managed conservatively as the urine output was adequate. On follow up, he attained spontaneous remission of nephrotic syndrome.

Conclusion

Overt proteinuria might be the manifestation of paraneoplastic syndrome in children with Hodgkin lymphoma and with the management of the primary disease, proteinuria resolves spontaneously.

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INTRODUCTION

An association between nephrotic syndrome and extrarenal neoplasia was described for the first time in 1922 [1]. Since then, a large number of cases have been published, few of them describing the link between Hodgkin lymphoma (HL) and nephrotic syndrome.

Though childhood nephrotic syndrome has an annual incidence ranging from 1.2 to 16.9 per 100,000 children, the incidence of nephrotic syndrome in Hodgkin lymphoma is less than 1% [2]. It was observed that nephrotic syndrome and Hodgkin's lymphoma may present clinically either simultaneously or within several months one after the other. The accurate basis of this relationship rests unknown, even though there have been hypotheses regarding a T-cell dysfunction. The early diagnosis of Hodgkin lymphoma is important as the secondary nephrotic syndrome needs only conservative measures.

Here, we present a 13 year old boy with Hodgkin lymphoma who had nephrotic syndrome as a paraneoplastic manifestation.

CLINICAL DESCRIPTION

A 13-year-old boy was admitted with complaints of abdominal pain, vomiting and loose stools for 2 months. There was a history of swelling of feet and periorbital puffiness noted for 6 weeks; despite which, he had significant weight loss of 5 kg within a couple of months (45 to 40 kg).

There wasn't any history of fever, bone pain, dyspnea/orthopnea, bleeding manifestations, decreased urine output or altered bowel habits. There was no significant medical illness in the past or contact with tuberculosis. On examination, there was no pallor and icterus. He had firm, non- tender right supraclavicular (4x4 cm) and bilateral lower cervical lymphadenopathy (3x5 cm), periorbital puffiness, ascites and bilateral pitting pedal oedema. His heart rate was 90/min, respiratory rate was 28/min and blood pressure of 100/60 mmHg. Abdomen was distended with no organomegaly and shifting dullness was noted suggestive of ascites. Other system examinations were unremarkable.

Due to the unusual features like weight loss despite edema and cervical lymphadenopathy in an adolescent boy, possibility of an underlying infection (tuberculosis) or malignancy (Hodgkin lymphoma) were considered in the background of nephrotic syndrome.

His complete blood counts, renal functions tests, liver functions tests were normal except hypoalbuminemia, 1.9 g/dL and hypercholesterolemia, 422 mg/dL (10.2mmol/L). Urine routine showed 3+ albuminuria, no hematuria, spot urine protein/creatinine ratio >2 and 24-hour urine protein was 2.4 g. Urine culture was sterile. Antinuclear antibodies, C3 and C4 were normal. Mantoux was not reactive. Chest X-ray was

normal. ESR was 24 mm. Cervical lymph node excision biopsy revealed loss of lymph node architecture, presence of Reed Sternberg cells, CD15 and CD30 staining which favoured nodular sclerosis type Hodgkin lymphoma (Figure 1). Bone marrow biopsy showed no infiltration by lymphoma. Whole body PET-CT done for staging showed cervical, mediastinal and para-aortic nodes, suggesting stage 3 disease. Secondary Nephrotic syndrome due to Hodgkin's lymphoma was made as a clinical diagnosis.

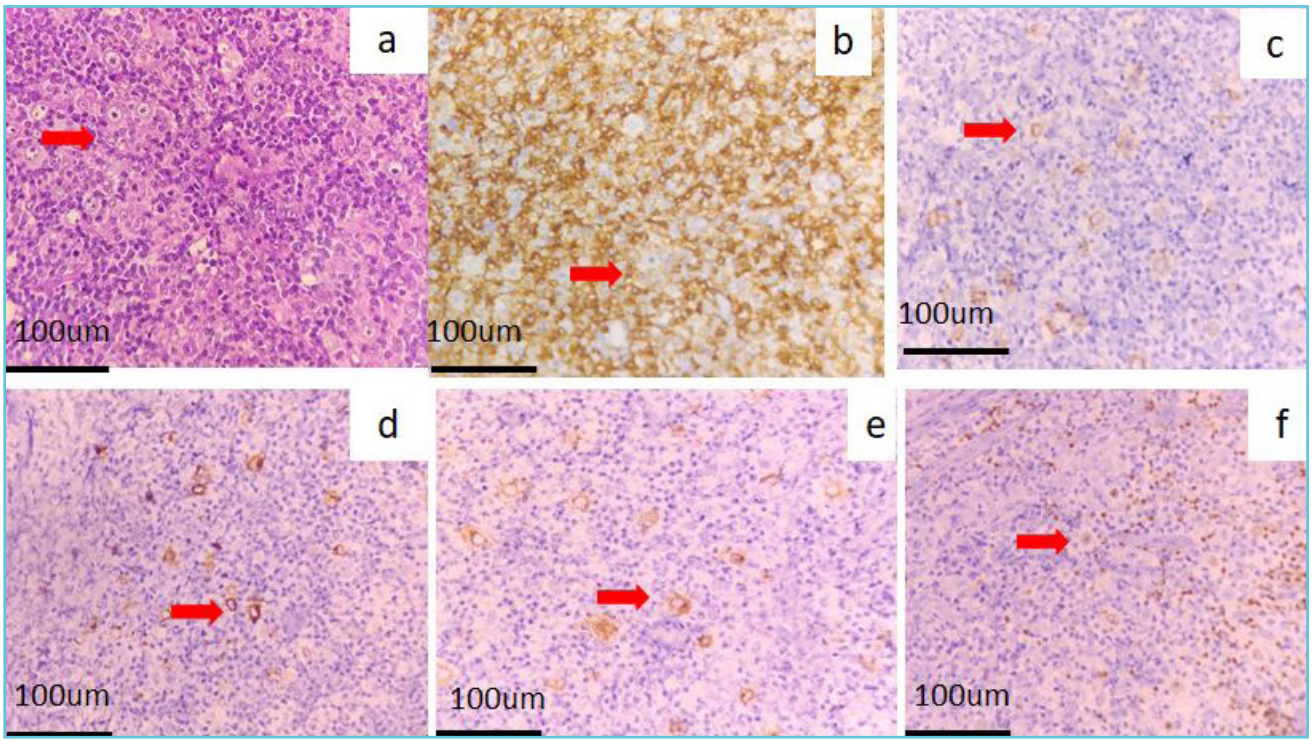
MANAGEMENT AND OUTCOME

He had been started on OEPA based chemotherapy (Prednisolone, Vincristine, Doxorubicin, Etoposide) as per EURONET Protocol for stage 3B Hodgkin lymphoma. He tolerated the chemotherapy well. Though he had symptomatic edema, he had been managed conservatively as the urine output was adequate. On follow up, he attained spontaneous remission of nephrotic syndrome with resolution of clinical edema in 2 weeks and normalisation of serum albumin (3.5 g/dL) along with absence of proteinuria by 5 weeks of chemotherapy (Figure 2).

DISCUSSION

Nephrotic syndrome is characterized by heavy proteinuria leading to edema, hypoalbuminemia 3 g/dL and hyperlipidemia (cholesterol > 200 mg/dL or 5.17 mmol/L). Heavy proteinuria is indicated by urine protein of 3+/4+ or spot urine protein/creatinine ratio of >2 or >50 mg/kg/day or > 40 mg/m^2/hour in a timed sample [3].

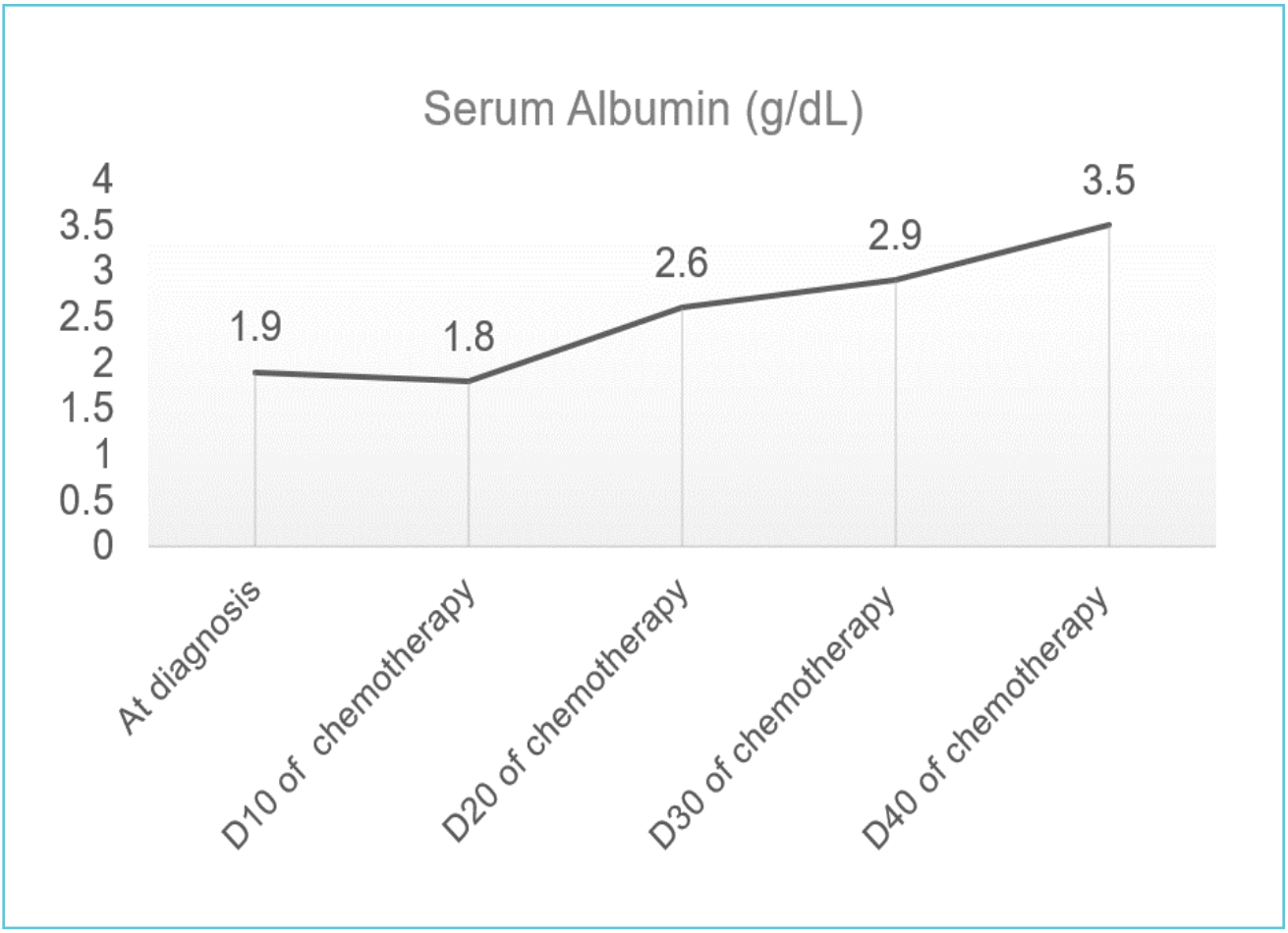
Figure 1 **Results of cervical lymph node excision biopsy**



Red arrows point to Reed Sternberg cells (RS).

(a) Hematoxylin and eosin stain 40x Large mononuclear RS cell with prominent nucleoli pointed by red arrow. (b) CD45 immunostain 20x Negative in RS cells. (c) EBV LMP immunostain 20x: RS cells were membrane positive. (d) CD15 immunostain 20x: RS cells were positive. (e) CD30 immunostain 20x: RS cells were positive. (f) PAX 5 immunostain 20X: RS cells are weakly positive (pointed by red arrow), reactive B cells are strongly positive.

Figure 2 Gradual rise in serum albumin level with chemotherapy



The kidney can be involved in neoplastic diseases in many ways: direct infiltration, renal vein thrombosis, renal artery or ureter compression by the neoplastic mass, tumour lysis syndrome, chemotherapy induced acute kidney injury, urinary tract infection, thrombotic microangiopathy and paraneoplastic disease. The intimate relationship between kidney and malignancy is bi-directional as oncology patients get various renal manifestations in due course and some nephrology patients who undergo kidney transplant, develop malignancies in the course of their treatment [4]. It is difficult to find the true incidence of glomerular disease caused by malignancy as kidney biopsies are rarely performed in patients with cancer.

Hodgkin and non-Hodgkin lymphomas, and acute myelogenous leukemia are the common malignancies associated with nephrotic syndrome in children [5]. Secondary nephrotic syndrome is thought to be a rare paraneoplastic syndrome of Hodgkin lymphoma (HL). The nodular sclerosis and mixed cellularity type of HL are the predominant histologic subtypes, in both adults and children. The incidence of nephrotic syndrome in Hodgkin lymphoma has been reported in the range of 0.6 to less than 1%. Stephan et al. analysed the prevalence of nephrotic syndrome in patients diagnosed with Hodgkin's lymphoma and found that 5 out of 483 children suffering from Hodgkin lymphoma, followed for a period of 13 years, developed nephrotic range

proteinuria [2]. Incidence of 0.6% was reported in a large series from Turkey [6]. In the majority of HL cases associated with nephrotic syndrome, selective albuminuria with normal renal function were the typical manifestation. From India, there were only 2 pediatric cases reported with HL and paraneoplastic syndrome. Both were neurological manifestation, one child with achalasia and Holmes Adie pupil [7] and another child with Ophelia syndrome [8].

Minimal change disease (MCD) is the most common associated pathological lesion with Hodgkin lymphoma. It is postulated that the cause of MCD could be T cell dysfunction with abnormal secretion of cytokines, altering the permeability of the glomerular basement membrane. Other previously reported glomerular diseases include membranous nephropathy, focal segmental glomerulosclerosis, mesangiocapillary glomerulonephritis, anti-glomerular basement membrane nephritis, and IgA nephropathy [9]. Mori reported a 15-year-old boy with frequently relapsing nephrotic syndrome with natural killer (NK) cell deficiency prior to the overt relapse of Hodgkin's disease. That child attained remission and complete recovery of the NK cell count after the treatment of the HL relapse [10]. Hence, proteinuria can be considered as the possibility of subclinical relapse of the lymphoma on follow up. The timing of nephrotic syndrome presentation in respect of HL varies in literature from months to few years [2].

CONCLUSION

Proper clinical examination is mandatory when facing a child with nephrotic syndrome, in order to exclude malignancies. Overt proteinuria might be the manifestation of paraneoplastic syndrome in children with HL and with the management of the primary disease, proteinuria resolves spontaneously. These children should be followed up as it is being observed that the relapse of the Hodgkin lymphoma causes consecutive relapse of the nephrotic syndrome. It emphasizes the importance of paediatrician, paediatric oncologists and nephrologists working together in managing these children.

LESSONS LEARNT

- Proper clinical examination gives us the lead to primary diagnosis in a child with oedema and proteinuria.
- Paraneoplastic syndrome resolves spontaneously in a child with malignancy when the primary disease is treated.
- Relapse of nephrotic syndrome in a child with Hodgkin lymphoma, might be the manifestation of relapse of malignancy.

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Author contributions

All authors were involved in the diagnosis and management of this case.

Consent

Informed and written consent was obtained from parent.

Ethical approval: Not applicable.

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Systemic pseudohypoaldosteronism type 1 due to a novel mutation in SCNN1B gene: a case report

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CASE REPORT

Pseudo hypoaldosteronism (PHA) is a type of channelopathy leading to life-threatening hyperkalemia, hyponatremia and metabolic acidosis in neonates. Type I PHA (PHAI) is characterized by either mutation in *NR3C2* (*MLR*) gene or genes related to subunit of ENaC channel, whereas Type II (A to E) PHA is due to mutations in other genes. Type I PHA is further divided into systemic and renal forms based on the gene and organ involved. Systemic PHAI is a rare, multisystem disease presenting as severe salt wasting in neonates. In this article, we report a case of systemic pseudohypoaldosteronism type 1 in a 2 days old neonate with a novel mutation involving *SCNN1B* gene. Our patient appears to be the first reported case of systemic PHAI due to *SCNN1B* mutation from India.

INTRODUCTION

Pseudo hypoaldosteronism (PHA) is a rare genetic disorder characterized by severe hyperkalemia associated with hyponatremia and metabolic acidosis. It is of two types: Type I PHA (PHAI), which can either be autosomal recessive or autosomal dominant and Type II (PHAII), further subclassified in A to E based on genetic etiology, all have an autosomal dominant mode of inheritance with type IID being inherited in autosomal recessive manner also.

Autosomal dominant PHAI results from mutation in mineralocorticoid receptor (*NR3C2* gene or *MLR* gene), whereas autosomal recessive PHAI results from mutation in any of the three subunits (alpha, beta, gamma) of epithelial sodium channels (ENaC)(1,2). Autosomal dominant subtype involves only the kidneys and is a milder form without systemic involvement, also known as renal PHAI. Autosomal recessive PHAI is a severe form, associated with multisystem involvement including kidneys, salivary glands, sweat glands, digestive glands and is called systemic PHAI. As a result of salt wasting from multiple systems, the patients develop renal manifestations like hyperkalemia, hyponatremia, pulmonary manifestations like wheezing, recurrent pulmonary infections, skin manifestations like miliaria, etc. (1,2). This disorder was first reported by Cheek and Perry in 1958 (3). Systemic PHAI is a very rare disease and only a few cases are reported to date. The estimated incidence of pseudohypoaldosteronism type 1 is around 1 in 80,000 individuals and that of systemic PHAI is 1 in 166,000 newborns (4,5).

Here we report a case of systemic pseudohypoaldosteronism type 1 in a neonate due to a novel variant detected in the gene *SCNN1B*.

CLINICAL CASE

A preterm (34 weeks) female neonate, full term, appropriate for gestational age with low birth weight, born of non-consanguineous marriage presented with thread like discharge from eyes and rashes all over the body on day 2 of life. The neonate had a history of sibling death due to sepsis and severe hyperkalemia at 28 days of life. Physical examination showed ropy discharge with crystallization of discharge from bilateral meibomian glands, miliaria all over the body with normal female genitalia and normal respiratory examination. (Figure 1)

Investigations revealed severe hyperkalemia (K^+ 6.7 mmol/L) and hyponatremia (Na^+ 131 mmol/L). Sepsis parameters were negative. A salt wasting disorder was suspected and further investigations were done. As history of a sibling death was present, congenital adrenal hyperplasia was suspected and 17 alpha hydroxyprogesterone levels were determined, which was normal. On day 4 of life, the patient became lethargic with increased secretions from eyes and increased skin rashes. Investigations revealed worsening hyperkalemia (K^+ 7.3 mmol/L), hyponatremia (Na^+ 129mmol/L), metabolic acidosis. A possibility of systemic pseudohypoaldosteronism type 1 was kept and investigations were done, revealing elevated renin and aldosterone levels.

Genetic testing was done on day 5 of life in view of systemic PHAI, which revealed a homozygous NM_000336.2:c.585+1G>A mutation in intron 3 of *SCNN1B* gene. This variant is not reported in 1000 genomes and has an allele frequency of 0.0004% in gnomAD database. The gene *SCNN1B* has a low rate of benign loss of function variants as indicated by a high LoF variants Z-Score of 2.68. The c.585+1G>A variant is a loss of function variant in the gene *SCNN1B*, which is intolerant loss of function variant, as indicated by the presence of existing pathogenic loss of function variant NM_000336.2:c.1542+1G>A and 4 others. There are 6 downstream pathogenic loss of function variants, with the farthest being 402 residues downstream of the variant

c.585+1G>A. Based on the above evidence, this variant has been classified as likely pathogenic according to ACMG guidelines (6) (Figure 2).

Sequencing of the protein coding regions in genes associated was performed using Illumina next generation sequencing (NGS) systems at a mean coverage of 80-100x in the target region. GATK best practice framework was followed for variant identification. BWA-mem aligner was used to align the obtain sequences to human reference genome (GRCh37/hg19). Duplicate reads identification and removal, base quality recalibration and re-alignment of reads based on indels were done. Sention's haplotypecaller module has been used to identify the variants which are relevant to the clinical indications (7). Along with this, deep variant analysis pipeline on Google cloud platform was used as a secondary pipeline to call genetic variants using inbuilt Sentieon modules (8). A total of 1425 genes

Figure 1 Pedigree chart

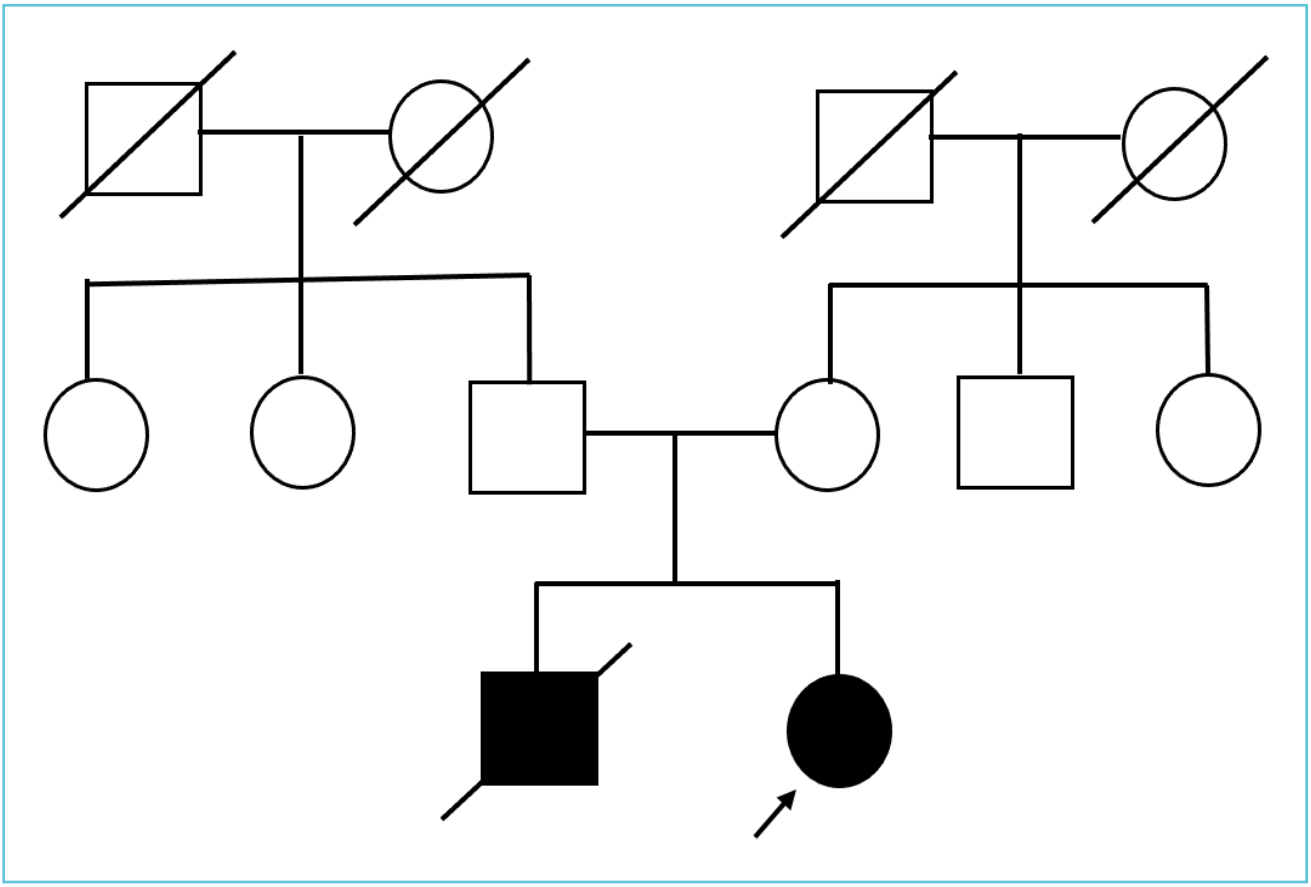

Figure 2 Next generation sequence findings of the case

Gene & Transcript	Variant	Location	Zygosity	Disorder (OMIM)	Inheritance	Classification
SCNN1B NM_000336.2	c.585+1G>A	Intron 3	Homozygous	Pseudohypoaldosteronism, Type I, Autosomal Recessive (264350)	Autosomal Recessive	Likely Pathogenic

related to the neonate's phenotype were covered. Quality checks (QC) were performed on all to exclude variants where sequencing is of poor quality. Additional QC metrics included total homozygous and heterozygous calls (SNVs and indels), proportion of variant calls that were common, number of variants falling into different annotated consequence categories, number of extreme heterozygotes (alternate allele proportion 0.8).

As the patient had worsening hyperkalemia and metabolic acidosis, insulin, $NaHCO_3$ and calcium polystyrene sulphonate were started. On day 24 of life, the patient had tachycardia, tachypnea, retractions and increased secretions, which was managed with chest physiotherapy, nebulization, O_2 therapy and antibiotics. Respiratory distress settled on day 29 of life. Oxygen requirement decreased and the patient was discharged on day 34 on sodium supplements, sodium bicarbonate, calcium polystyrene sulphonate, nebulization with N-acetyl cysteine. The patient was on regular follow up. Eye discharge was persisting with persistent miliaria but no signs of respiratory distress. On day 70 of life, the patient went into sudden cardiac arrest at home. The cause was attributed to severe hyperkalemia but no laboratory investigations could be done to confirm the cause of death (Table 1).

DISCUSSION

Pseudo hypoaldosteronism type 1 was described first by Cheek and Perry in 1958. It is further of two types, namely renal PHAI and systemic PHAI. Renal PHAI occurs due to mutation in mineralocorticoid receptor (MLR). It is a milder

Table 1 Laboratory investigations of the case

Laboratory parameter	Day of life 2 (admission)	Day of life 5	Day of life 17	Day of life 34 (discharge)
Sodium (mmol/L)	131	129	141	140
Potassium (mmol/L)	6.7	7.3	5.5	4.2
Urinary sodium (mmol/L)	472			
Urinary potassium(mmol/L)	9			
17-alpha hydroxyprogesterone (ng/ml)		3.9		
Plasma Renin (µIU/ml)		267.2		
Aldosterone (ng/dL)		1065		
Clinical exome (Next generation sequencing)		*SCNN1B* mutation		

variant as only kidneys are involved. Systemic PHAI is a more severe type characterized by a defect in any of the three subunits (alpha, beta, gamma) of ENaC sodium channels. Aldosterone, the principal mineralocorticoid of human body acts through mineralocorticoid receptors (MLR) and ENaC channels. ENaC is expressed in multiple tissues: sweat glands, meibomian glands, colon, salivary glands, etc. Hence, its mutation leads to multisystem involvement. Due to the impaired action of aldosterone, the renal regulation of sodium and potassium is disturbed leading to a triad of hyperkalemia, hyponatremia, and metabolic acidosis.

Systemic PHA type 1 is an autosomal recessive disease caused due to mutation in any of the three genes, namely *SCNN1A* (chromosome 12p13.31), *SCNN1B* (chromosome 16p12.1) or *SCNN1G* (chromosome 16p12.1). The risk increases in neonates born as a result of consanguinity (inbreeding). However, there was no inbreeding in our case. But there was an history of similar illness in sibling leading to death. Further genetic analysis of parents is required to know the carrier status.

To date, only about 35 cases of systemic pseudohypoaldosteronism have been reported around the world and only 2 cases in India. Out of which, the majority are due to mutation in SCNN1A gene and less than 10 cases are due to *SCNN1B* gene mutation. No case of *SCNN1B* mutation has been reported from India.

Most of the cases of systemic pseudohypoaldosteronism were reported in neonatal age groups, mostly in first two weeks of life. Neonates present with severe salt wasting manifestations, growth failure, letharginess and is associated with high mortality rates. As the age increases, salt wasting episodes tends to decrease and the patient might be asymptomatic and may lead a healthy life.

Investigations should include serum electrolytes, urinary electrolytes and a blood gas. Further investigation shows increased levels of serum aldosterone and renin activity. Genetic analysis can be done for confirmation of the disease and for any novel mutations.

Management includes sodium supplementation, combating hyperkalemia with albuterol nebulization, insulin, calcium gluconate infusion (with ECG monitoring), potassium binders and sodium bicarbonate for metabolic acidosis. Patients generally require lifelong treatment. As the age increases, the salt wasting manifestations decreases and patient may lead a normal healthy life.

LEARNING POINTS

- When a neonate presents with severe life-threatening hyperkalemia, a possibility of systemic pseudohypoaldosteronism should be kept.
- Systemic PHAI has high mortality rate when presented during infancy and mortality, morbidity decreases as age increases.
- Treatment includes management of hyperkalemia, hyponatremia and metabolic acidosis. Prompt treatment might lead to the survival of patient and may lead a healthy life.

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Contributions

Kamal Joshi: Data collection and primary manuscript.

Prashant Kumar Verma: Finalising manuscript.

Manidipa Barman: Preparing primary manuscript

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Protect your fibroblasts before they become gametes

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LETTER TO THE EDITOR

About 5 years ago, we wrote a science-fiction essay describing the creation of a super-talented tennis player (carrying the imaginary name Rofa Nadofederal) by using stem cells of two great tennis champions, Rafael Nadal and Roger Federer (1). In a subsequent communication, we drew attention to tennis players and other athletes, not to share their towels with the audiences after the competition is completed, because fibroblasts, which are abundant in their sweat, could be used to generate gametes. It is conceivable that these gametes could then be unlawfully used for reproductive purposes (2). These hypothetical scenarios have now come much closer to becoming a reality. In a September 2021 paper in Science (3), Yoshino et al reported generation of ovarian follicles (eggs and supporting ovarian tissue) by using only mouse pluripotent stem cells. These functional mouse eggs, that

could be fertilized in a dish, are capable of forming mouse embryos. The primary goal of this new advance was to develop new treatments for infertility. A review of the rapid progress in this field, including human in-vitro gametogenesis has been recently published (4).

This new work confirms that it is now possible, at least in the mouse, that offspring could be created by reprogramming of only fibroblasts. In humans, fibroblasts obtained without consent, or even the knowledge of the donors, could, in the near future, be harvested from sweat and used to produce offspring with a unique genetic make-up, and presumably abilities, like the ones described for Rofa Nadofederal (1). We suggest, in view of the latest results (3), that it is now time for people of all walks of life to start paying more attention as to who, and under which circumstances, could get possession of their easily assessable fibroblasts, like those from sweat. We expect that this issue will gain paramount importance if and when a human offspring is created solely by using reprogrammed fibroblasts. It seems that this evolving technology is only a few years away from implementation in humans (4). The huge ethical implications of these new reproductive possibilities are obvious. At present, the best defense against such activities seems to be intense vigilance as to who could have access to your easily obtainable fibroblasts, such as those from sweat.

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Authors' disclosures

Authors declare no potential conflict of interest.

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