Evaluation of Current Clinical Research Spectrum of Sickle Cell Anemia to Approach New Innovative Developmental Techniques for Enhancing Sudanese Health Systems

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Abstract

Background

Sickle cell Anemia and/or Disease (SCA/SCD) is an autosomal recessive disease leading to abnormal hemoglobin with global prevalence in general and tribal burden in sub-Saharan Africa particularly.

Methods

Recent patients' assessments were done using routine diagnostic protocols for SCA monitoring and manifestations. Insight of the physicians towards innovative techniques at the molecular level were done to enhance medical performance of the disease investigations. The online questionnaire showed the response and acceptance levels of the physicians to introduced innovative techniques in integration with current clinical and laboratory's spectrums. Data interpretation and statistical analysis were done using IBM-SPSS ver 25 and MS. Excel 2019.

Results

Clinical manifestation showed painful crises, (Hand and food syndrome) and enuresis were observed in more than half of the patients, while stroke observed only in 5.5%. A strong association was observed in males regarding Hb vs RBC and MCV vs HbA2.However. females show an association between MCV vs HbA2 only. SCA current spectrum include CBC, BF and Solubility Sickling Test showed significant results in Hemoglobin $P < 0.05$ and Strong association was observed between Hb vs RBC, MCV vs HbA2 for males and MCV vs HbA2 for female only. Polymerase Chain Reaction (PCR) was introduced to detect inherited polymorphic traits in infants and parents beside the flow cytometry gave a detailed informatic image for the up normal blood cells shape and amounts comparing with normal ones. The high response of the online questionnaire (100%) showed the attitude of doctors and related staff accepting innovative techniques for enhancing current health system.

Conclusions

Although the current clinical and laboratory diagnostic protocols are effective for diagnosing SCA, the follow-up and treatment but the approach of innovative techniques is highly demanded for detecting un-seen symptoms and hidden polymorphic traits, beside monitoring all patients with including other family members.

Introduction

Sickle cell anemia is an autosomal recessive disease, which is globally distributed; however, it causes devastating effects in sub-Saharan Africa (1–3). Historically, the first case of sickle cell anemia reported

in Sudan in 1926 and considered as the first case in Africa (4). In 1950, the first case of HbS gene was reported in Sudan (5). Later studies showed that sickle cell gene frequencies vary from region to another in Sudan as well as within the same region The prevalence rate of sickle cell anemia in Sudan ranging from 2 to 30.4% (6–8). The disease is a major health problem in certain parts of the country, particularly the western region. 'e HbS allele frequently exists among the Misseriya tribe, and it is estimated to range from 18.2% in Kordofan to 30.4% in Darfur(5, 9–12). In Khartoum, the capital of Sudan, where patients reported to Khartoum Teaching Hospital, 5.1% and 0.8% of children were HbAS and HbSS, respectively(11). The basic mechanism for Sickle-cell anemia is the mutation in position 6 of the β-globin chain in amino acid valine replacing glutamic acid, leading to hemoglobin polymerization. As a result, all manifestations and complications of the disease develop. Subsequently the shape of the red blood cells changed to biconcave disc, then continuous hemolysis and chronic anemia with jaundice. The abnormal shape of the red blood cell leads to obstructions to micro vasculature, causing vas -occlusive crisis. More recent work has correlated polymorphisms in the MBL_2 gene to Vaso-occlusive events in children with sickle cell anemia. (13–16). Genotypes related to lower/intermediate levels of serum mannose-binding lectin (MBL) have been associated with a higher frequency of painful episodes, the variation and activation of the complement system by the lectin pathway influence the sickling process remains to be better characterized(17–19). By means of entirely infections in the sickle cell patients, malaria degrades the earlier anemia. Malaria was found to be the cause of most severe anemia in hospitalized patients (20). The latter achieved innovation in the managing of sickle cell disease came in the 1980s, through the finding that a drug named hydroxyurea could diminish discomfort for patients living with the disease. Beyond that, efforts have been focused mostly on prevention, rather than treatment or early detection for people already living with the disease. Many elements work together or individually such as infection, dehydration, stress to precipitate complications. Meticulous follow up, vaccinations, and the preventive measures could improve the quality of life for such patients(21). Novel healing policies might be divided into; pathophysiology-related new treatments and innovations in remedial beneficial options such as hematopoietic stem cell replacement and gene remedy. Novel therapeutic methods that use medications to upgrade the downstream sequelae of HbS polymerization have not verified to be as active as hydroxyurea (HU) which has an “anti-sickling” consequence on induction of embryonic hemoglobin (HbF, α2γ2). In addition to great advances in HSCT and gene therapy, new pharmacological anti-sickling approaches have developed beside introducing up to date molecular based technologies (22, 23).

Justifications

There is a conscious between Sudanese doctors as well as medical students, the sickle cell disease is common only among certain tribes, which might lead to miss diagnosis of many cases among unknown tribes, more over the use of electrophoresis which will only be positive after 6 months might add to the confusion

Objectives

To evaluate the present clinical, hematological, and biochemical spectrum protocols of sickle cell anemia among Sudanese children and introduce new updated diagnostic systems for more accurate medical assessment to introduce innovative developmental techniques for investigating sickle cell disease among Sudanese children.

Patients And Methods

Study design

This is a long-term retrospective, descriptive and comparative hospital-based study. Data and information were collected for patients who presented to Al Buluk hospital which is a teaching hospital in Omdurman, Sudan. Blood samples were collected from patients presented with sickle cell crises. In addition, the perception and opinion of doctors was taken through a structured questionnaire in consideration for the best tool to diagnose sickle cell anemia, the questioner was filled through online and derived from literature(24, 25).

Sample size

This was calculated according to World Health Organization program of sample size calculation version 2.0 (Appendix – 1)(26, 27). From previous studies, the prevalence of sickle cell anemia (HbAS + HbSS) is 10%, with alpha 0.05 and error margin of 5%(26, 27).

Inclusion criteria

The study was conducted between January 2017–2022. A total of 113 patients with SCA, aged 4 months to 12 years were included and 100 physicians were requested to the fill the questionnaire to reflect their opinion about current and innovative methods for investigation sickle cell anemia.

Exclusion criterion

1- sickle -thalassemic disease. 2- sickle cell trait. 3- Positive sickling test only. 4- sickle cell in stable state

Patients Process and procedures

All admitted patients with sickle cell crises were re-examined by a pediatrician, then after handling the patient management started with addressing patients needed and complete hemogram, renal function test, liver function test and other investigation and admission for further management to the pediatrics words(28–30).

Sickle cell anemia clinical diagnosis

Blood tests were checked for the form of hemoglobin that underlies sickle cell anemia. If the patient/ child has sickle cell anemia, other tests might be suggested to check for complications of the disease. If the patient/ child carries the sickle cell gene, he will be referred to a genetic counselor.

Sickle cell anemia laboratorial investigation

Many techniques and protocols were used for the detection and monitoring of the sickle disease. These methods can be separated into two focal types

1. presently used techniques in the analysis of SCD
2. innovative techniques which are still in the research phase.

Several assessments have been updated associated to the advance of point of care (POC) SCD detection(31).

Current Techniques Used to Diagnose, Monitor and Compare SCD Hemoglobin:

1. **Complete Blood Cell Count**: The complete blood count (CBC) is a key test to characterize the diverse categories of anemia. Nevertheless, the hemoglobin mutation drive impact on the hematological restrictions and screening an adjustable modification.
2. **Peripheral Blood Smear**: The peripheral blood smear (PBF) is usually done after spotting abnormality in the automation counts and is considered a landmark of any hematological evaluation. PBF examines the morphology of the blood cell and evaluates any microscopic changes, which can provide valuable information that helps in the diagnoses of the diverse types of anemia.
3. **Solubility Sickling Test**: Sickling examinations are built on the polymerization of HbS in the deoxygenated state. The solubility assessment is the most used nowadays; its principle is based on the insolubility of Hb-S in the occurrence of concentrated phosphate buffer, a hemolyzing agent, and sodium dithionate. These mediators crystalize the HbS and precipitate the cells, which divert the light and cause solution turbidity and the product compared with negative and positive controls (32).
4. **Hemoglobin Electrophoresis**: Electrophoresis is a form of chromatography methods, and it is considered as one of the significant tests used to distinguish Hb variants. In this assessment, an electrical field is applied to enable the migration of electrically charged molecules. Towards classifying hemoglobin variations, dissimilar pH and mediums were employed, moreover citrate agar at acidic pH or cellulose acetate electrophoreses at alkaline pH. Alkaline electrophoresis is an analytical tool that has been used to distinguish thalassemia and sickle cell anemia at pH 8.4. First, a hemolysate is prepared from the red blood cells; then, it is added to a cellulose strip and run-in buffer at a constant voltage in an electrophoresis chamber(33).

Innovative Techniques to develop SCD Clinical Research:

(These are the methods to be recommended in according to the response of the physician in the questionnaire)

1. **Isoelectric Focusing**: Isoelectric focusing (IEF) is a high-resolution technique for separating proteins depends on their isoelectric points (pI). The Hb particles moved through a pH slope until they spread

their isoelectric points where the left charge is zero. The Hb molecules precipitate and looks like a sharp band. (34).

2. **Polymerase Chain Reaction (PCR)-Based Techniques**: Polymerase chain reaction is one of the most powerful diagnostic techniques, where special enzymes are used to amplify specific parts of the genetic materials to millions of copies, using specific primers. PCR can distinguish recognized single genes or numerous genes in a solitary tube. Then, the result can be detected by gel electrophoresis, sequencing, melting curve analysis, or monitoring the change in the fluorescence(35). For detection of mutation point or minor loss of DNA, the suitable technique is amplification-refractory mutation system (ARMS). The ARMS value is to use primers with exact sequences to permit the amplification of DNA in the occurrence of the marked allele. Consequently, the recognition of the aimed allele is built on the occurrence of the PCR product. The alleles can then be differentiated on agarose gel with different band sizes(36–38).
3. **Flow Cytometry**: Conventional flow cytometry techniques have been used to detect sickle cells based on fluorescent markers or cellular morphology. Innovative flow cytometry created on imaging methods has been established to improve the sensitivity by combination of cell population investigation and blood cell structural data. Developing an imaging flow cytometry assay (SIFCA) and software algorithm to differentiate between sickle RBCs and normal RBCs based on their morphology. SIFCA is performed by diluting the peripheral blood sample, deoxygenating the cells by reducing the oxygen to 2% for 2 h, and then analyze it using imaging flow cytometry. Finally, the cells are classified based on the morphology into sickled and normal cells by using algorithm software(39).

Statistical analysis

Analysis was done by using SPSS version 21 where p-value and correlation were calculated for the hematological and biochemical data. The standard criteria for descriptive analysis a percentage, mean, standard, and Chi-square test. Significance is set as p-value of < 0.05 variables.
Ethical approval: Approval was taken from the Research Committee of Al Buluk hospital

Results And Findings

Records of 113 patients diagnosed with sickle cell anemia according to Hb electrophoresis, of which only 91 records were considered complete regarding history, examination, investigations, and management. Out of which 40 patients were males and 51 were females (study population calculated by software appendix 1). Painful crisis was encountered in 65 patients (71.4%), dactylitis (hand and foot syndrome) in 51 patients (56%), while enuresis was reported in 47 patients (51.6%) and stroke in 5 patients (5.5%) (Fig 1).

Sickle cell anemia clinical assessments outcomes:

- Sickle cell disease is a caused of variety different illnesses, the most common polymorphic trait among Sudanese is sickle cell anemia HbSS with the genotype βs/βs.
- Other forms of the SCD are formed with a combination of βS mutation with other HBB mutations, such as sickle-hemoglobin C disease (HbSC) and sickle-β-thalassemia either HbSβ+ or HbSβ0 (β0 means there is no βglobin synthesis, while β+ means reduced production of β-globin) have been also observed.
- The maximum severe types of SCD are HbSS and HbSβ0, and they demonstrated similar clinical picture. HbSC and HbSβ+ are considered the fewer severe types of SCD.

The clinical image of the sickle-β-thalassemia:

- The disease ranges from asymptomatic to severe state similar to HbSS sickle cell anemia, while, in some HbSC cases, severe and life-threatening complications may appear.
- Some genetic factors can modify the sickle cell's clinical expression when co-inherited with the βS gene, such as β-globin gene mutations, either one-gene removal or two-gene removal.

Sickle cell anemia laboratorial findings:

Complete Blood Cell Count outcomes:

- Patients with homozygous SS and heterozygous S/ β mutations usually present with hemolytic anemia where the red blood cells (RBCs), hemoglobin and hematocrit are in insignificant amounts. Dissimilarity, the totals of white blood cells (WBC) and platelet are raised, and they can vary accordingly.
- Reticulocyte counts were variable and depend on varied factors such as the degree of anemia caused by the cell's hemolysis, sequestration, and bone marrow response to anemia. In SCD patients receiving hydroxyurea, Mean Corpuscular Volume (MCV) is typically raised.
- Elevated red cell distribution width (RDW) was seen in SCD patients because of RBCs' different subprop Latinos. Although CBC was widely used to describe the hematological parameter as valuable information, it was insufficient to give a complete picture of patients' diagnoses.

Blood smears observations:

- In sickle cell anemia, moderate to severe Latinos is seen with a variable number of elongated sickle cells, which is best observed when the red blood cells are deprived of oxygen.
- The preparation of blood smear slides is quite simple, quick, and cheap. Even though marginal blood smear is a useful hematological assessment, it depends on the pathologist's skills, and the

accessibility of qualified pathologists is inadequate. Additionally, the blood film investigation is also difficult according to the variations in the cell's edge, position, form, and magnitude.

Table 1: Hematological and biochemical comparative spectrum

Laboratory profile	Males Mean±SD	Females Mean±SD	P value
Mean Hb	8.34±1.72	7.23±1.82	0.58
Mean Corpuscular Hemoglobin Concentration	32.22±4.15	32.43±3.28	0.05
Mean Packed Cell Volume	25.33±7.11	25.83±6.17	0.05
ESR >30	7	11	0.05
Mean platelets	260±6.15	261±5.95	0.05
Hb F	18.79±5.21	16.86±6.76	0.05
Hb S	None	None	

N= 91

Solubility Sickling Test:

- This test is easy to execute and low-cost. It suffers from a false-negative result when utilized for newborns, due to the presence of a high volume of hemoglobin F and when the HbS is less than 10% of the total hemoglobin. Moreover, false-negative results are detected in patients with coinheritance of β-thalassemia trait and severe anemia.
- In contrast, false positives are detected in patients with high serum viscosity, erythrocytosis, highly marked leukocytosis and in some cases of anemia. Furthermore, the sickle solubility assessments cannot distinguish between sickle cells trait (SCT) and SCD, and they are indifferent to the recognition of hemoglobin AS (HbAS. These weaknesses make them tough to be used in screening programs.

Table 2: Correlation between laboratory profile in males and females:

Laboratory profile correlation	Males		Females	
	r	p	r	p
MCV vs HbA2	0.7231	0.0023	0.8693	<0.0002
Hb vs MCV	0.018	0.94	0.3743	0.0753
MCV vs HbA2	-0.7952	0.02	-0.1354	0.3335
MCV vs HbF	0.3087	0.69	-0.1673	0.76
Hb vs RBC	0.019	0.002	-0.2321	0.002

Hematological profile showed significantly high ESR>30 ml/hour in 13 patient (14.3%), with p-value 0.001, and MCV> 84.5 with p-value 0.003 (Table 1). Positive correlation between MCV vs HbA2 in both males and females. a positive correlation was observed in Hb vs RBC for both males and females (Table 2)

Hemoglobin Electrophoresis advantages:

- Consequently, the diverse hemoglobin forms with dissimilar net charges are divided into several bands depending on their movement.
- Hemoglobin electrophoresis can distinguish between HbS and HbC, which are the most clinically important alternatives.
- Nevertheless, electrophoresis does not differentiate between hemoglobin alternatives with the similar electrical charges and gives the identical relocation forms, such as HbD and HbG, which comigrate with HbS; HbE and Hb0-Arab have comparable relocation to the HbC molecules.
- Additionally, alkaline electrophoresis can be pretentious by the occurrence of great quantities of hemoglobin F in infants, which can control the slighter electrophoresis band. So, additional attention should be occupied to consistently notice the HbS.
- Furthermore, minor bands such as HbA2, HbH, and Hb Bart's might be lost. Consequently, extra competent test must be used as an investigative test to overwhelm these restrictions. Citrate agar electrophoresis is executed in acidic pH 6.0–6.2, and it depends on the interface of the agaropectin in the gel mixture with the mechanical variations of the Hb. Most hemoglobin variations that comigrate at alkaline pH can be divided successfully using citrate agar electrophoresis.
- Citrate agar electrophoresis is not affected by the high amount of hemoglobin F in newborns; thus, it can be used as a diagnostic test for sickle cell disease at birth.
- Nevertheless, it is difficult and exciting to achieve in inadequate incomes areas. Capillary electrophoresis has been recognized to separate Hb segments and identify sickle cell disease and thalassemia. The capillary electrophoresis splits the protein in an unprocessed fused-silica column constantly.

Evaluation of Innovative techniques to be introduced in current research for enhancing health systems

Isoelectric Focusing

- This method can detect HbS and HbA easily in a high concentration of HbF. Moreover, it separates Hb D-Punjab from HbS. Commonly, it can deliver the outcome in 45 min.
- Though IEF is costly and needs highly trained staffs to interpret the outcomes due to the larger number of bands, it is still considered the standard test for neonatal screening, as it needs a small volume of sample and can be used with a dried blood spot.
- Nevertheless, in the occurrence of diverse Hb variations, Capillary Zone Electrophoresis (CZE) is better than HPLC for enumerating HbA2 excepting in the incidence of HbC.
- Furthermore, a fully automated Neonate Fast Hb device with Isoelectric Focusing cord blood mode can analyze dried blood spots on filter paper and liquid cord blood. Therefore, it can be used in the newborn screening test. These compensations make the Isoelectric Focusing tool the first-line test for screening hemoglobinopathies in neonatal and grownup patients.

Polymerase Chain Reaction (PCR)-Based Techniques

- PCR sensitivity and specificity have reformed the prenatal and newborn investigative field. Quite a lot of PCR-based methods are recognized to detect βs mutations, for instance high-resolution melting (HRM) analysis, which is simple, subtle, and cost-effective for usage in mass screening of SCD genotypes.
- Additional simple, low-cost PCR-based method has been settled using bi-directional allele-specific amplification (ASA) and a hot star system to provide more specific single-tube genotyping, where the point mutation of sickle cell anemia is applied as the SNP model. Furthermore, discriminatory conditions have enabled the determination of homozygous and heterozygous conditions created on the dissimilar band dimensions on the agarose gel electrophoresis.
- ARMS has been frequently applied in prenatal analysis by finding of sickle cell mutation in the embryonic sample. The ARMS's sensitivity has been measured by associating the outcome to recognize the occurrence of hemoglobin variations by HPLC. Meanwhile demonstration of allele-specific oligonucleotide (ASO) hybridization to detect sickle cell mutation using two PCR primers can also be applied. Where single primer was used for the ordinary allele and the additional one for the mutated allele.
- The primer is combined to the complementary sequence and amplified, which in turn releases the fluorescent label that determines the amount of the target. This technique can distinguish between the allelic difference.

Flow Cytometry:

- 100 images were analyzed for normal cells and 100 images of sickle cell for comparison, and they reported 100% sensitivity and 99.1% specificity.
- SIFCA can assess sickling tendency in SCA patients to identify the severity of the disease and drug monitoring.
- A developed in-vitro photoacoustic flow cytometry (PAFC) for morphological detection of sickle cells containing hemoglobin S. It employed photothermal and photoacoustic spectra for determination of hemoglobin heterogeneity and accumulation of the HbS in sickle RBCs.
- The sickled RBCs presented 2–4-fold lesser linear means than regular RBCs. This technique is suitable in monitoring the sickling conditions to progress microfluidic flow cytometry created on the electrical resistivity spectroscopy.
- This method notices the variations in the electrical resistance caused by the change in the cells' shape from the round soluble cells to sickle firm cells under hypoxic disorder.
- The control cells were gained from a healthy benefactor and the tested cells from sickle cell patients, and the modification in the electrical resistivity was measured to display the variance among regular cells and sickled cells.
- They showed that the electrical impedance signal can be used as an indicator of the cell sickling events. However, it is still unclear if these novel flow cytometry techniques can be used to monitor disease severity or if they can distinguish between sickle cell trait and sickle cell disease (40).

Acceptability of Innovative Techniques Based on Clinical Professional Questionnaire:

100 participants filled Google forms online, they assayed all survey specimens by the analytical schemes they routinely use and report for each specimen the presumptive phenotype, presumptive clinical assessment, and any additional clinical classifications considered dependable with their diagnostic results and program processes.

Recommended Screening Quality Assurance Program Proficiency

Screening program using online methods was done to maintain high-quality results to ensure accurate physicians responses for their acceptance to introduce new diagnostic techniques in integration with their regular used ones (Table 3).

Table 3: Multi-Level Schemes to enhance the physicians' point of view for SCD screening (N= 91)

Method	Clinical Manifest	CBC, BF	Isoelectric Focusing	PCR	Flow Cytometry	HPLC	Monitoring techniques
Level A: complete selection	12%	11%	68%	65%	27%	13%	
Level B: Partial selection	23%		49%		11%		
Level C: All positive	51%				44%		31%
Level D: All negative				35%	29%	6%	21%

*Advanced portable point of care techniques has been developed to provide a low-cost, simple, and user-friendly device for detecting SCD, for instance coupling solubility tests with portable devices, using smartphone microscopic classifications, image processing techniques, rapid immunoassays, and sensor-based platforms.

Discussion

Although many studies reflect clinical pattern and profile in sickle cell anemia. Therefore, specialized medical care focusing on prevention and regular assessment of disease management is done to reduce morbid events as well as the mortality rate beside the laboratory patterns as well. The sample size in the current study was more than the sample size in the previous studies in Sudan it contains patients and physicians, which might explain the high ratio in the current study. Sickle cell disease referral clinics in the country in general and the capital in particular allow one third of the population to carry out regular follow ups, provide health education and prescribe folic acid, hydroxy urea as well as prophylactic antibiotics(41). The clinical profile and complications between males and females with respect to hematological profile were with insignificant difference(42, 43) . A strong association was observed between Hb vs RBC and MCV vs HbA2in males and MCV vs HbA2 in females. As a result, a statistical computerized system has been developed to provide a more accessible way to recognize the type of anemia (Table 1), a significant difference was found among males and female in hematological findings reflected in mean packed Cell Volume, ESR < 0,005. Strong correlation was observed between MCV vs HbA2, Hb vs RBC for both sexes, however correlation between MCV vs HbA2 for male only. Though it might be difficult to blame the gene for this difference, similar findings were reported in Brazil, but differ from the Indian study. This gender difference might reflect the frequency of reports to hospital, and only community-based study can give a clear image regarding gender differences(44-46). The prevalence of enuresis is high among Sudanese children, many factors contribute to the prevalence of enuresis among children like toilet training, developmental delay, socioeconomic status which are common to all children including sticklers due to the involvement of the kidneys due to the pathogenesis of sickle cell disease. Enuresis was found in more than half of the patients in contrast to previous studies done locally in Sudan and abroad, which were 38% and 43.9%, respectively(12, 47). Stroke was reported in 5.5 % of the patients

in the current study with similar results reported from Yemen, however, in the absence of community-based study and brain imaging, the exact rate of stroke cannot be determined(48). Obviously, the clinical image showed more than half of the patients with sickle cell anemia presented with dactylitis which is consistent with findings among sticklers in the Kingdom of Saudi Arabia and differs from finding among American Sickler's. Many factors contribute to such complications like the pattern of the disease and ethnicity(49, 50). The complete blood count (CBC) is a main test to describe the diverse forms of anemia. However, the hemoglobin mutation will affect the No significant changes were observed between males and females regarding the hematological values, which go along with studies performed in India and Gnana, however significant correlation in MCV vs HbA2 in both males and females was observed. MCV and HbA2 in the current study showed significance as well as negative correlation in males in contrast to females (Table 2), which partially agreed with Shrikhande et al. MCV was significantly high in our patient in contrast to previous studies and agreement with Nigerian study done by Juwah(51-53). Blood smears must not be used for hemoglobinopathy screening. A smear is undependable as it needs the occurrence of sickled cells which may or may not be in flow at the time of group. Blood smears also cannot differentiate homozygous from heterozygous nor can they detect other hemoglobin variants (32). The invalidating impact of SCD on patient survival, quality of life and cost for health systems require the development of new therapeutic options to treat sickle cell related acute and chronic complications. Several techniques and assays are used for the detection and monitoring of the sickle disease(54). These methods can be separated into two focal groups: (1) presently used systems in the analysis of SCD; and (2) innovative methods which are frequently still in the research phase. Many articles have been published associated to the progress of point of care (POC) SCD findings. PCR is a simple technique to be established initially for providing rapid prenatal diagnosis to the couples with known sickle cell mutation(22, 55, 56). The sensitivity of ARMS-PCR can be improved by using appropriate procedures to distinguish maternal cell DNA contamination. Alternative simple, low-cost PCR-based procedure has been developed using bi-directional allele-specific amplification (ASA) and a hot star technique to deliver additional exact single-tube genotyping, wherever the point modification of sickle cell anemia is used as the SNP model. Fertrin et al have developed an automated, observer-independent sickling assay by applying imaging flow cytometry, which allows software algorithm-driven classification of 20,000 RBCs per sample, yielding a less subjective and more high-throughput assay. This assay has been shown to sensitively detect the known effects of both patient-dependent and - independent variables, such as HbF concentration and sample pH(57). High-performance liquid chromatography (HPLC) and Gas Chromatography are used for accurate quantification of normal and variant hemoglobin at low concentrations, enabling differentiation of Hb S/β+-thalassemia from sickle cell trait (Hb A/S), as well as a quantitative description of compound heterozygous disorders such as Hb S/HPFH (hereditary persistence of fetal hemoglobin. They are not recommended because does not definitively distinguish Hb S/β°-thalassemia from Hb S/S (identification requires DNA testing or integration with other laboratory studies) and also high cost(51, 58). Eventually, the physicians' response at the online questionnaire obviously declares the importance of introducing innovative new techniques in integration with the current used ones as shown in (Table 3) an (Figs 2-6).

Conclusion

This study surely will draw the attention of the concern physician and policy makers for adopting new techniques for definite precise diagnosis and research development of sickle cell anemia, the study recommends that Innovative methods for proper diagnosis and clinical research of sickle cell anemia should be considered urgently and badly needed to minimized the suffering of parents and their children who suffer the disease.

Declarations

Area for improvement

Being a hospital-based study, the results cannot be generalized unless it involves many hospitals in the region or at the community level with longitudinal follow up.

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References

1. Elmahdi M, Fadalla T, Suliman M, Elsayed M, Awad Elhaj AM, Hussein H. Moyamoya syndrome and stroke among pediatric sickle cell disease patients in Sudan: A cross-sectional study. Ann Med Surg (Lond). 2022;78:103815.
2. Runkel B, Kluppelholz B, Rummer A, Sieben W, Lampert U, Bollig C, et al. Screening for sickle cell disease in newborns: a systematic review. Syst Rev. 2020;9(1):250.
3. Burnham-Marusich AR, Ezeanolue CO, Obiefune MC, Yang W, Osuji A, Ogidi AG, et al. Prevalence of Sickle Cell Trait and Reliability of Self-Reported Status among Expectant Parents in Nigeria: Implications for Targeted Newborn Screening. Public Health Genomics. 2016;19(5):298–306.
4. Talha M, Osman B, Abdalla S, Mirghani H, Abdoon I. Pediatric Sickle Cell Disease in Sudan: Complications and Management. Anemia. 2022;2022:3058012.
5. Daak AA, Elsamani E, Ali EH, Mohamed FA, Abdel-Rahman ME, Elderdery AY, et al. Sickle cell disease in western Sudan: genetic epidemiology and predictors of knowledge attitude and practices. Trop Med Int Health. 2016;21(5):642–53.
6. Mahjoub SA, Abdelrhman E, El-Deen ME, Mustafa MS, Ali EW. Angiotensin-converting enzyme insertion/deletion polymorphism is not associated with vasoocclusive complications of sickle cell anemia. Int J Appl Basic Med Res. 2016;6(4):267–70.

7. Bitoungui VJ, Pule GD, Hanchard N, Ngogang J, Wonkam A. Beta-globin gene haplotypes among cameroonians and review of the global distribution: is there a case for a single sickle mutation origin in Africa? OMICS. 2015;19(3):171–9.
8. Salih NA, Hussain AA, Almugtaba IA, Elzein AM, Elhassan IM, Khalil EA, et al. Loss of balancing selection in the betaS globin locus. BMC Med Genet. 2010;11:21.
9. Adam MA, Adam NK, Mohamed BA. Prevalence of sickle cell disease and sickle cell trait among children admitted to Al Fashir Teaching Hospital North Darfur State, Sudan. BMC Res Notes. 2019;12(1):659.
10. Sabahelzain MM, Hamamy H. The ethnic distribution of sickle cell disease in Sudan. Pan Afr Med J. 2014;18:13.
11. Mohammed AO, Attalla B, Bashir FM, Ahmed FE, El Hassan AM, Ibnauf G, et al. Relationship of the sickle cell gene to the ethnic and geographic groups populating the Sudan. Community Genet. 2006;9(2):113–20.
12. Bayoumi RA, Abu Zeid YA, Abdul Sadig A, Awad Elkarim O. Sickle cell disease in Sudan. Trans R Soc Trop Med Hyg. 1988;82(1):164–8.
13. Eaton WA. Impact of hemoglobin biophysical studies on molecular pathogenesis and drug therapy for sickle cell disease. Mol Aspects Med. 2022;84:100971.
14. Bao X, Zhang X, Wang L, Wang Z, Huang J, Zhang Q, et al. Epigenetic inactivation of ERF reactivates gamma-globin expression in beta-thalassemia. Am J Hum Genet. 2021;108(4):709–21.
15. Musicki B, Burnett AL. Mechanisms underlying priapism in sickle cell disease: targeting and key innovations on the preclinical landscape. Expert Opin Ther Targets. 2020;24(5):439–50.
16. Alli LA, Nnodu OE. Anti-sickling Efficacy of Methanolic Extract of Medicinal Plants from Gwagwalada, Nigeria. West Afr J Med. 2020;37(7):790–5.
17. Figueiredo CVB, Santiago RP, da Guarda CC, Oliveira RM, Fiuza LM, Yahouedehou S, et al. Priapism in sickle cell disease: Associations between NOS3 and EDN1 genetic polymorphisms and laboratory biomarkers. PLoS One. 2021;16(2):e0246067.
18. Batista J, Arcanjo GS, Batista THC, Sobreira MJ, Santana RM, Domingos IF, et al. Influence of UGT1A1 promoter polymorphism, alpha-thalassemia and beta(s) haplotype in bilirubin levels and cholelithiasis in a large sickle cell anemia cohort. Ann Hematol. 2021;100(4):903–11.
19. Nunes K, Aguiar VRC, Silva M, Sena AC, de Oliveira DCM, Dinardo CL, et al. How Ancestry Influences the Chances of Finding Unrelated Donors: An Investigation in Admixed Brazilians. Front Immunol. 2020;11:584950.
20. Sumbele IUN, Teh RN, Nkeudem GA, Sandie SM, Moyeh MN, Shey RA, et al. Asymptomatic and sub-microscopic Plasmodium falciparum infection in children in the Mount Cameroon area: a cross-sectional study on altitudinal influence, haematological parameters and risk factors. Malar J. 2021;20(1):382.
21. Juwah AI, Nlemadim EU, Kaine W. Types of anaemic crises in paediatric patients with sickle cell anaemia seen in Enugu, Nigeria. Arch Dis Child. 2004;89(6):572–6.

22. Detemmerman L, Olivier S, Bours V, Boemer F. Innovative PCR without DNA extraction for African sickle cell disease diagnosis. Hematology. 2018;23(3):181–6.
23. Anie KA, Paintsil V, Owusu-Dabo E, Ansong D, Osei-Akoto A, Ohene-Frempong K, et al. Organ damage in sickle cell disease study (ORDISS): protocol for a longitudinal cohort study based in Ghana. BMJ Open. 2017;7(8):e016727.
24. Adigwe OP, Onavbavba G, Onoja SO. Attitudes and practices of unmarried adults towards sickle cell disease: emergent factors from a cross sectional study in Nigeria's capital. Hematology. 2022;27(1):488–93.
25. Adeodu OO, Alimi T, Adekile AD. A comparative study of perception of sickle cell anaemia by married Nigeria rural and urban women. West Afr J Med. 2000;19(1):1–5.
26. Badawy SM, Cronin RM, Hankins J, Crosby L, DeBaun M, Thompson AA, et al. Patient-Centered eHealth Interventions for Children, Adolescents, and Adults With Sickle Cell Disease: Systematic Review. J Med Internet Res. 2018;20(7):e10940.
27. Tluway F, Urio F, Mmbando B, Sangeda RZ, Makubi A, Makani J. Possible Risk Factors for Severe Anemia in Hospitalized Sickle Cell Patients at Muhimbili National Hospital, Tanzania: Protocol for a Cross-Sectional Study. JMIR Res Protoc. 2018;7(2):e46.
28. Johnson KM, Jiao B, Bender MA, Ramsey SD, Devine B, Basu A. Development of a conceptual model for evaluating new non-curative and curative therapies for sickle cell disease. PLoS One. 2022;17(4):e0267448.
29. Reeves SL, Dombkowski KJ, Madden B, Cogan L, Liu S, Kirby PB, et al. Considerations When Aggregating Data to Measure Performance Across Levels of the Health Care System. Acad Pediatr. 2022;22(3S):S119-S24.
30. Paintsil V, Amuzu EX, Nyanor I, Asafo-Adjei E, Mohammed AR, Yawnumah SA, et al. Establishing a Sickle Cell Disease Registry in Africa: Experience From the Sickle Pan-African Research Consortium, Kumasi-Ghana. Front Genet. 2022;13:802355.
31. Maier-Redelsperger M, Labie D, Elion J. Long-term hydroxyurea treatment in young sickle cell patients. Curr Opin Hematol. 1999;6(2):115–20.
32. Arishi WA, Alhadrami HA, Zourob M. Techniques for the Detection of Sickle Cell Disease: A Review. Micromachines (Basel). 2021;12(5).
33. Srinivasan R, Eugene Christo VR, Nambannor Kunnath R, Katare P, Venukumar A, Nambison NKM, et al. Optical absorbance-based rapid test for the detection of sickle cell trait and sickle cell disease at the point-of-care. Spectrochim Acta A Mol Biomol Spectrosc. 2022;279:121394.
34. van Zwieten R, Veldthuis M, Delzenne B, Berghuis J, Groen J, Ait Ichou F, et al. Hemoglobin analyses in the Netherlands reveal more than 80 different variants including six novel ones. Hemoglobin. 2014;38(1):1–7.
35. Abdulwahab H, Aljishi M, Sultan A, Al-Kafaji G, Sridharan K, Bakhiet M, et al. Whole blood transcriptomic analysis reveals PLSCR4 as a potential marker for vaso-occlusive crises in sickle cell disease. Sci Rep. 2021;11(1):22199.

36. Nezhad FH, Nezhad KH, Choghakabodi PM, Keikhaei B. Prevalence and Genetic Analysis of alpha- and beta-Thalassemia and Sickle Cell Anemia in Southwest Iran. J Epidemiol Glob Health. 2018;8(3–4):189–95.
37. Nadkarni AH, Gorakshakar AC, Sawant PM, Italia KY, Upadhye DS, Gorivale MS, et al. The phenotypic and molecular diversity of hemoglobinopathies in India: A review of 15 years at a referral center. Int J Lab Hematol. 2019;41(2):218–26.
38. Gupta AD, Nadkarni A, Mehta P, Goriwale M, Ramani M, Chaudhary P, et al. Phenotypic expression of HbO Indonesia in two Indian families and its interaction with sickle hemoglobin. Indian J Pathol Microbiol. 2017;60(1):79–83.
39. van Beers EJ, Samsel L, Mendelsohn L, Saiyed R, Fertrin KY, Brantner CA, et al. Imaging flow cytometry for automated detection of hypoxia-induced erythrocyte shape change in sickle cell disease. Am J Hematol. 2014;89(6):598–603.
40. Ozpolat T, Chang TC, Wu X, St John AE, Konkle BA, Chen J, et al. Phenotypic analysis of erythrocytes in sickle cell disease using imaging flow cytometry. Cytometry A. 2022;101(5):448–57.
41. Al-Nood HA. Thalassaemia and glucose-6-phosphate dehydrogenase deficiency in sickle-cell disorder patients in Taiz, Yemen. East Mediterr Health J. 2011;17(5):404–8.
42. Valentine SL, Bembea MM, Muszynski JA, Cholette JM, Doctor A, Spinella PC, et al. Consensus Recommendations for RBC Transfusion Practice in Critically Ill Children From the Pediatric Critical Care Transfusion and Anemia Expertise Initiative. Pediatr Crit Care Med. 2018;19(9):884–98.
43. Xu JZ, Garrett ME, Soldano KL, Chen ST, Clish CB, Ashley-Koch AE, et al. Clinical and metabolomic risk factors associated with rapid renal function decline in sickle cell disease. Am J Hematol. 2018;93(12):1451–60.
44. Nwabuko OC, Onwuchekwa U, Iheji O. An overview of sickle cell disease from the socio-demographic triangle - a Nigerian single-institution retrospective study. Pan Afr Med J. 2022;41:161.
45. Nnajekwu UC, Nnajekwu CO, Onukwuli VO, Uwaezuoke NA, Ezenwosu OU, Ikefuna AN, et al. Relationship between disease severity and folate status of children with sickle cell anaemia in Enugu, South East Nigeria. Afr Health Sci. 2021;21(2):759–64.
46. Wilson TT, Chou SC, Becker S, Schuur JD, Beaudoin F. Evaluation of sex disparities in opioid use among ED patients with sickle cell disease, 2006–2015. Am J Emerg Med. 2021;50:597–601.
47. Salih KMA. The impact of sickle cell anemia on the quality of life of sicklers at school age. J Family Med Prim Care. 2019;8(2):468–71.
48. Al-Saqladi AM, Maddi DM, Al-Sadeeq AH. Blood Transfusion Frequency and Indications in Yemeni Children with Sickle Cell Disease. Anemia. 2020;2020:7080264.
49. Choi S, O'Neil SH, Joshi AA, Li J, Bush AM, Coates TD, et al. Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome. Am J Hematol. 2019;94(10):1055–65.
50. Helton KJ, Glass JO, Reddick WE, Paydar A, Zandieh AR, Dave R, et al. Comparing segmented ASL perfusion of vascular territories using manual versus semiautomated techniques in children with

sickle cell anemia. J Magn Reson Imaging. 2015;41(2):439–46.

51. Baig MA, Swamy KB, Baksh AD, Bahashwan A, Moshrif Y, Al Sawat A, et al. Evaluation of role of HPLC (Merits & Pitfalls), in the diagnosis of various hemoglobinopathies & thalassemic syndromes. Indian J Pathol Microbiol. 2021;64(3):518–23.
52. Jaber RZ, Hassan MK, Al-Salait SK. Microcytosis in children and adolescents with the sickle cell trait in Basra, Iraq. Blood Res. 2019;54(1):38–44.
53. Kotila TR. Beta thalassaemia in Nigeria: myth or fact? Afr J Med Med Sci. 2013;42(4):355–8.
54. Nakagawa A, Cooper MK, Kost-Alimova M, Berstler J, Yu B, Berra L, et al. High-Throughput Assay to Screen Small Molecules for Their Ability to Prevent Sickling of Red Blood Cells. ACS Omega. 2022;7(16):14009–16.
55. Gutierrez M, Shamoun M, Seu KG, Tanski T, Kalfa TA, Eniola-Adefeso O. Characterizing bulk rigidity of rigid red blood cell populations in sickle-cell disease patients. Sci Rep. 2021;11(1):7909.
56. Guruprasad P, Mannino RG, Caruso C, Zhang H, Josephson CD, Roback JD, et al. Integrated automated particle tracking microfluidic enables high-throughput cell deformability cytometry for red cell disorders. Am J Hematol. 2019;94(2):189–99.
57. Fertrin KY, Samsel L, van Beers EJ, Mendelsohn L, Kato GJ, McCoy JP, Jr. Sickle Cell Imaging Flow Cytometry Assay (SIFCA). Methods Mol Biol. 2016;1389:279–92.
58. Oluwole EO, Adeyemo TA, Osanyin GE, Odukoya OO, Kanki PJ, Afolabi BB. Feasibility and acceptability of early infant screening for sickle cell disease in Lagos, Nigeria-A pilot study. PLoS One. 2020;15(12):e0242861.

Figures

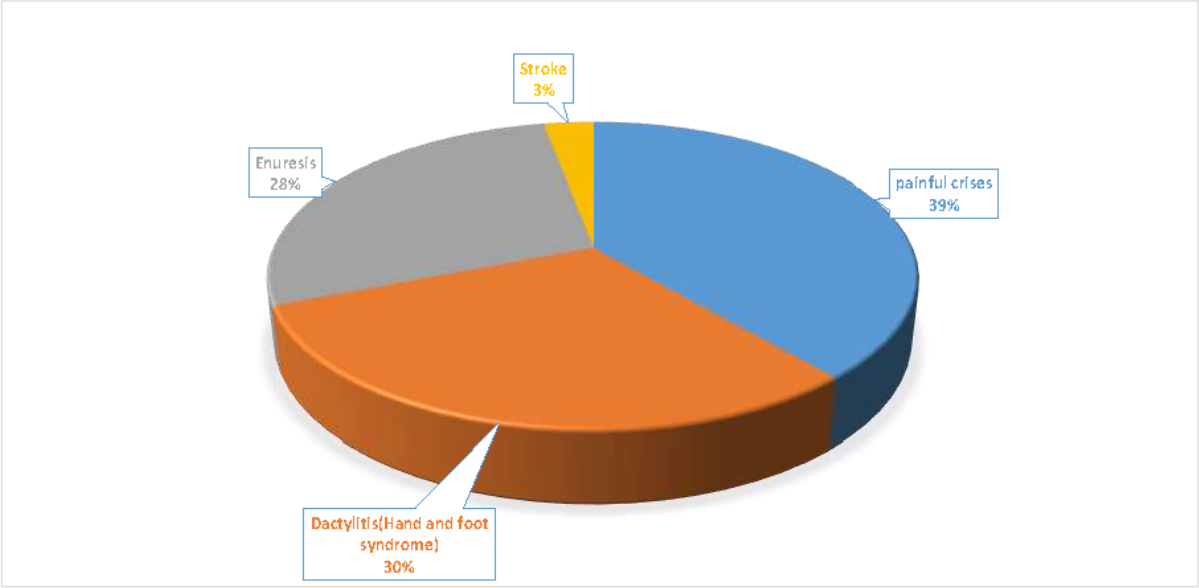

Fig 1: Clinical manifestation spectrum (N= 91)

Figure 1

See image above for figure legend.

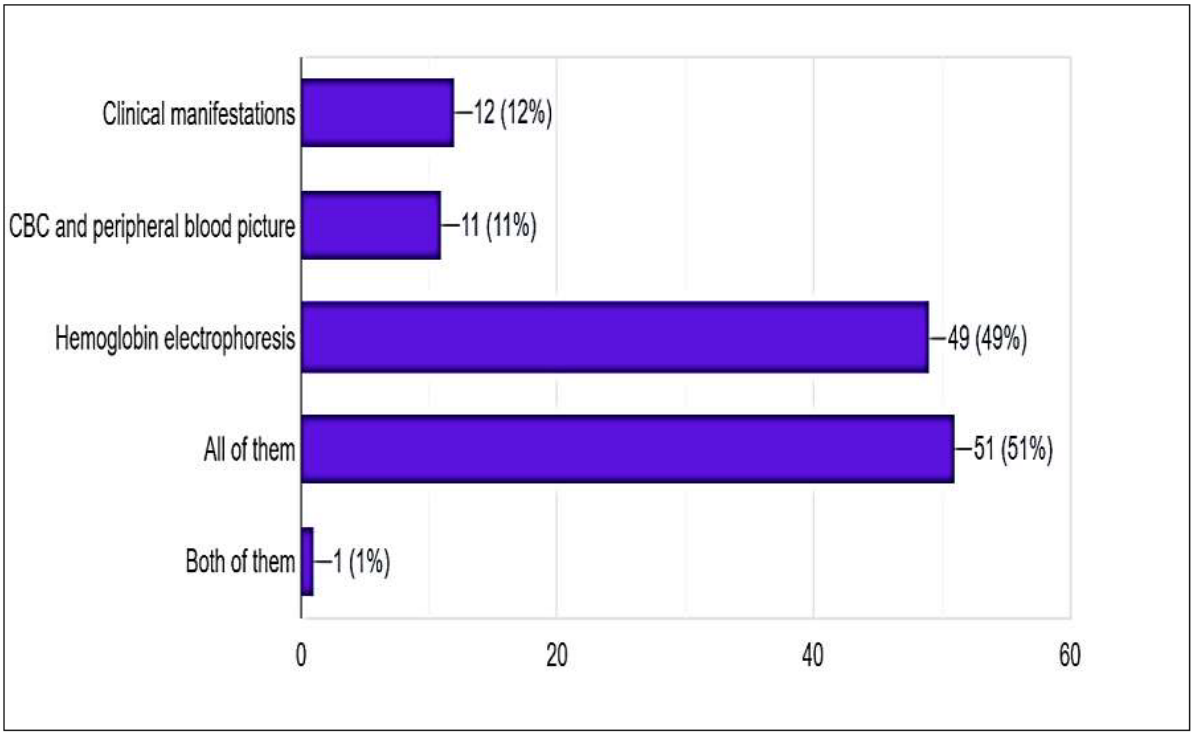

Fig 2: Current clinical and laboratory diagnostic spectrum (N= 91)

Figure 2

See image above for figure legend.

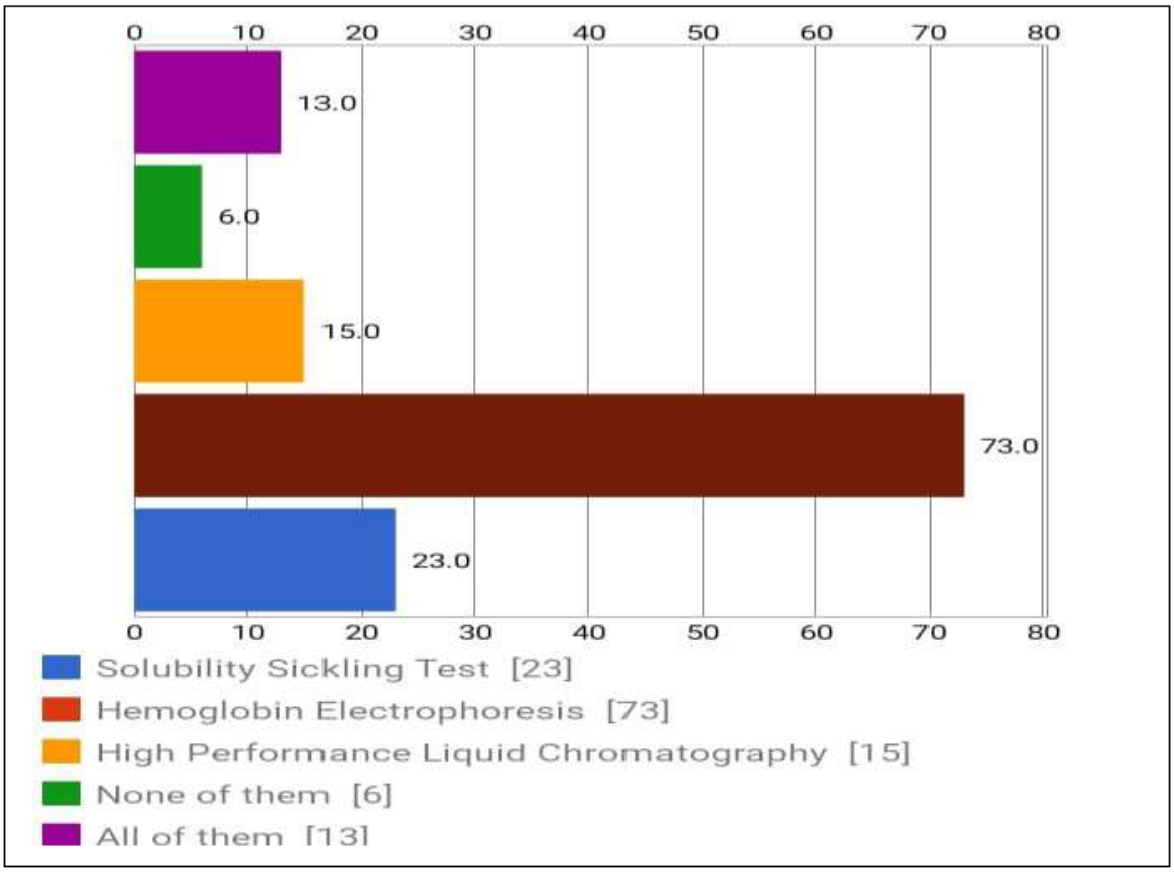

Fig 3: Current clinical monitoring spectrum (N= 91)

Figure 3

See image above for figure legend.

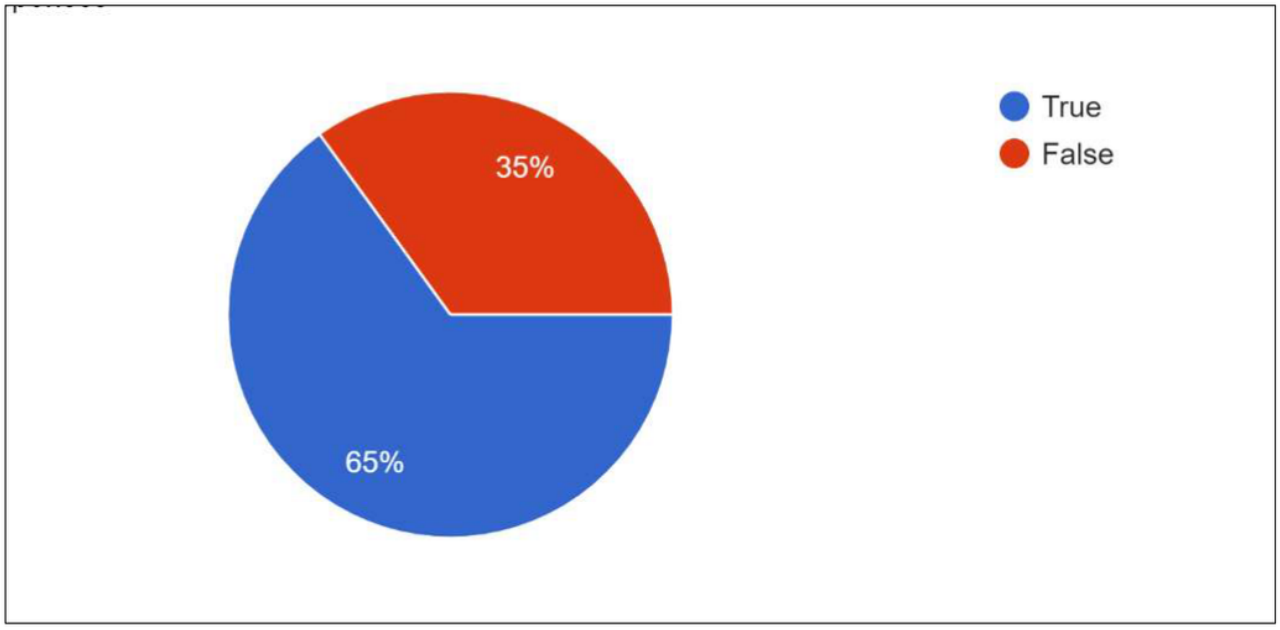

Fig 4: Acceptance of PCR as integrated diagnostic tool (N= 91)

Figure 4

See image above for figure legend.

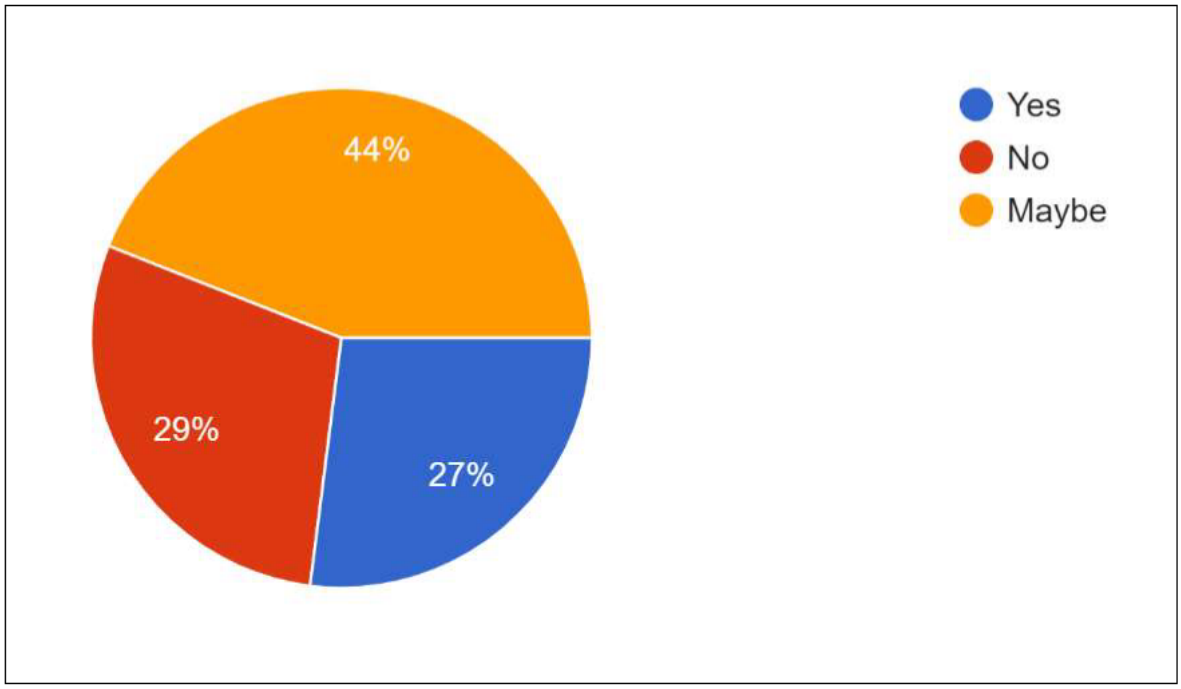

Fig 5: Acceptance of innovative diagnostic tool (Flow Cytometry) (N= 91)

Figure 5

See image above for figure legend.

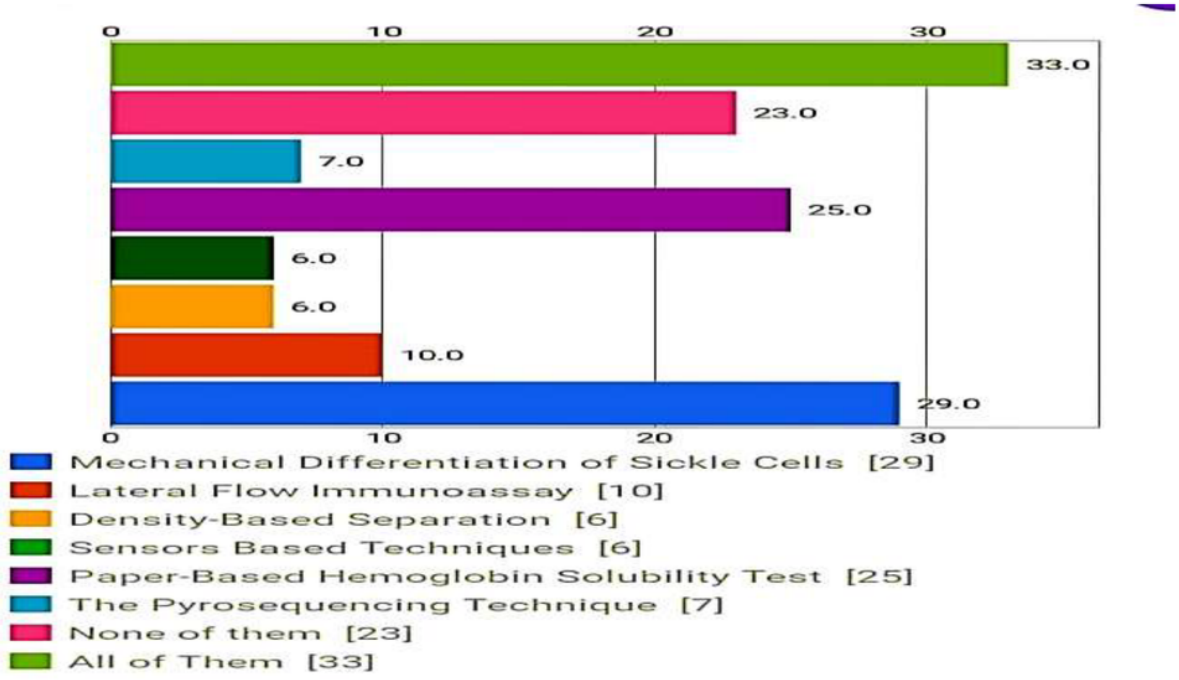

Fig 6: Acceptance of innovative monitoring techniques (N= 91)

Figure 6

See image above for figure legend.

Supplementary Files

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