## Abstract

A seven7 -year- -old boy ~~come up~~presented ~~with~~ complaining of abdominal pain-. The given case report illustrates ~~the~~ a case of anaemia ~~which~~ that, after investigation, ~~later turned out to be the~~was found to be a case of sickle-cell anaemia ~~when it was studied in detail~~. Many investigations of blood were ~~also made like~~undertaken, including a complete blood count, haemoglobin electrophoresis test ~~as also conducted~~ and blood film examination. ~~According to t~~These initial investigations ~~initially~~ showed~~,~~ ~~there was~~ a decline in haemoglobin ~~(Hb)~~ count, red blood cell~~s~~ (RBC) count and haematocrit ~~(Hc)~~, ~~whereas,~~and an increase in mean cell haemoglobin ~~(MCH)~~ and mean corpuscular haemoglobin concentration ~~(MCHC)~~ levels ~~were found to be increased~~. Mean corpuscular volume ~~(But, MCV)~~ was in the normal range~~s according to the test report~~. ~~Red blood cells (RBC) not only~~In addition to ~~appeared~~ appearing ~~a less number~~fewer, the ~~but they~~ RBCs ~~also~~ exhibited anisochromasia, and the~~ir shape was abnormal, they appeared~~ y had an abnormal ~~as~~ sickle- -shaped and ~~had a~~ boat- -shaped appearance, with polychromasia. The first diagnosis ~~which came up~~was of ~~revealed~~ anaemia, but the differential diagnosis also ~~includes~~ included sickle-cell/β°β thalassemia and sickle-cell/-haemoglobin C disease. In this case history, clinical examination and the case presentation delivered a great deal of knowledge ~~in~~ for diagnosing the disease. ~~When~~Upon detailed ~~the~~ investigation~~s were carried out in detail, it showed~~a strongly positive result was found ~~with~~ in the sickle solubility ~~test~~ and sickling test~~s~~. A strong haemoglobin S was also found ~~in~~ using alkaline electrophoresis, which ~~also~~ confirmed~~s~~ sickle-cell disease.


## Introduction

The clinical history is helpful in most ~~of the~~ cases of h~~H~~aemoglobinopathy; it is a very useful tool in correct diagnosi~~sing it correctly - k~~. Knowing the patient's~~a~~ family history and ethnicity is also essential. ~~Other than this p~~Preliminary haematological test~~s, like~~ such as approximating the concentration of haemoglobin (Hb) and red blood cells ~~(red blood cells~~RBCs), also play a crucial role. Similarly, a ~~through~~ comprehensive clinical examination of properly -stained peripheral blood smear~~s is also gives us the key~~provides necessary knowledge for diagnosis. Other diagnostic test~~s, like~~ such as quantitation of Hb-A2 and Hb-F, the ~~and~~ sickle solubility test, Hb~~Haemoglobin~~ electrophoresis ~~or~~ and high-performance liquid chromatography, must also ~~needs to~~ be ~~carried out~~conducted. Some other tests for



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**Commented [CE2]:** Please confirm that this was your intended meaning.

**Commented [CE3]:** Note that abstracts are considered separate from the body of the text for the purpose of defining abbreviations. This means that any abbreviations defined in the abstract will need to be defined again at their first instance of use in the body of the text. For this reason, any abbreviations not used again in the abstract were deleted here. They are instead defined in the body of the text, except where they were only used once again, in which case they are written out in full.

**Commented [CE4]:** Please check whether this was when RBCs were high, not low.

**Commented [CE5]:** Please check that this was what you meant to say.

**Commented [CE6]:** As this abstract is considered separate from the body of the text, please make sure that you introduce the topic of your paper fully here, without relying on the abstract.

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~~knowing~~ determining the levels of ferritin in blood, total iron binding capacity and ~~levels of~~ iron levels ~~is~~ are also important to supplement the iron deficiency.

Sickle-cell anaemia manifests in childhood ~~so it~~and is ~~one of those~~a disease ~~which~~ that can easily be diagnosed in its early stages. The disease process ~~initiates~~ begins with severe anaemia exhibiting some clinicopathological features ~~causing~~ and results in obstructed~~obstruction of the~~ vessels due to the sickle-shaped ~~red blood cells~~RBCs ~~leading to~~causing ~~the~~ tissue~~s~~ infarction. Vaso-occlusion occurs as a result of leukocyte~~s~~ recruitment and the coagulatory and inflammatory mediators of the sickle cells ([1~~2~~]). Extravascular haemolysis is the outcome of chronic haemolysis ([2~~1~~]).

The polymeri~~s~~zation of the sickle-shaped haemoglobin (HbS) due to the mutant ~~haemoglobin~~ Hb formed by the substitution of glutamic acid by valine in position six of the beta chain is the key pathophysiology involved in the disease process. ~~These~~ This abnormal ~~haemoglobin~~ Hb become~~s~~ poorly soluble in the deoxygenated state and undergoe~~s~~~~s~~ polymeri~~s~~zation ~~on~~ under various ~~occasions~~conditions, including ~~like~~ the concentration of HbS, temperature, hypoxia acidosis and ~~different~~ factors such as 2,3-diphosphoglycerate concentration (2,3-DPG)~~.~~ .The aggregation of HbS lead~~s~~ ~~to~~ the RBCs ~~become~~ to become rigid and less deformable. The repeat cycle of oxygenation, polymeri~~s~~zation and sickling~~,~~ dis~~t~~rupt~~s~~ion ~~of~~ cationic haemostasis, ~~occur where~~resulting in loss of water, $Ca^{2+}$ ~~+~~ and $K^{k+}$ from the cells . The living capacity of erythrocytes is based on the quantity of HbF and level of membrane damage. ~~The s~~Sickle-shaped cells have a shorter life-span, ~~of~~ at around 20 days ~~where as the~~compared to 120 days for normal RBC~~s~~ ~~have survival duration of 120 days~~. The anaemia in sickle-cell disease is haemolytic anaemia. ~~The p~~Patient~~s~~ ~~who is~~ with homozygous ~~haemoglobin~~ HbS ~~, has~~exhibit the abnormal synthesis of beta chain~~s~~.~~,~~ T~~t~~here is a lack of HbA~~-~~, a different percentage of ~~haemoglobin~~ HbF, ~~and~~ a small percentage of ~~haemoglobin~~ HbA2 ([2~~1~~, 3) and a small proportion~~s~~ of HbS.

~~There is acute painful episode because of T~~the constriction~~s~~ of vessels, ~~which is~~ a very common symptom in patients with sickle-cell anaemia, causes an acute painful episode.~~ It~~



**Commented [CE9]:** The meaning here is not clear. Do you mean 'to provide supplementary information concerning iron deficiency'?

**Commented [CE10]:** Please check whether you need to provide references for any of the information in this paragraph.

**Commented [CE11]:** Note that I have edited your references according to your university's Vancouver guidelines, including the use of parentheses instead of brackets or superscript. Your reference numbering was corrected so that the first source cited is numbered 1, the second source cited is numbered 2, and so on. Please check each of these changes carefully to ensure that the correct source is still being pointed to.

**Commented [CE12]:** Do you mean high temperatures? Please check.

**Commented [CE13]:** Please check that you agree that this should be written in this way.
Otherwise, you might just write this as calcium and potassium.

**Commented [CE14]:** Is this the best place for this sentence? It does not seem to follow smoothly from the sentences before. Please consider whether it would be better to move this sentence to somewhere else in the paragraph.

**Commented [CE15]:** Higher or lower?

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~~is a common finding in young adults whereas it's less prevalent in elderly patients.~~ The painful episode is more frequent when~~—~~ patients have low levels of HbF~~—~~ and alpha thalassemia with higher baseline ~~haemoglobin~~ Hb levels. ~~( )~~ ~~It~~Painful episodes are ~~is~~ a common finding in young adults, ~~whereas it's~~but are less prevalent in elderly patients.

In ~~majority of~~most ~~the~~ cases, there is no identifiable cause, but pain episodes ~~can~~ may be triggered by a cold, infection, menses, dehydration ~~and~~ or stress. ~~It is noticed that i~~In young patients, pain episodes appear~~s~~ as dactylitis or hand-foot syndrome,~~—~~ in which there is swelling of the dorsal surface of the hands and feet. This swelling subsides within ~~1–2~~one to two weeks. R~~The r~~adiography ~~shows~~ reveals thinning of the cortex and some degenerative changes in the affected bones~~.~~. In older children and adults,~~–~~ the ~~commonest~~ most common sites of pain are the chest, abdomen, back and~~–~~ extremities.

Some ~~of the lab~~laboratory tests ~~which were performed~~ to detect the presence of sickle cells were performed on blood film~~.~~. Hb electrophoresis is a powerful tool ~~in~~ for determining~~e~~ the sickling of Hb,~~—~~ to distinguish between the heterozygous state and other variants of the ~~haemoglobin~~ Hb band. ~~The a~~Almost all ~~of~~ the ~~haemoglobin~~ Hb ~~being~~ is ~~haemoglobin~~ HbS and ~~the absence of~~ HbA is absent ~~(~~the HbF band is difficult to see).

**Commented [CE17]:** Please insert the missing reference.

**Commented [CE18]:** Here, I think you are referring to the painful episode, rather than to sickle-cell anemia more broadly. Please check, and clarify if necessary.

**Commented [CE19]:** These two paragraphs were joined together as they appear to be on the same topic (painful episodes)

**Commented [CE20]:** 'Of sickle-cell anemia', or 'of the onset of a painful episode'. Please clarify here.

**Commented [CE21]:** Please check that this was what you meant to say. Did you mean to include a figure of a radiograph image here? Please insert the figure immediately after this paragraph, and insert a cross-reference (see Figure 1) at the end of this sentence.

**Commented [CE22]:** Again, please check whether you need to provide references for the information in this paragraph.

**Commented [CE23]:** This sentence was incomplete. These edits have made the sentence grammatically complete. However, please check that this was your intended meaning.

**Commented [CE24]:** This sentence is grammatically correct, but it does not follow logically from the sentences before it. The main idea in this paragraph is not clear. The relationship between the sentences is also unclear. Please review this section and rewrite for clarity. This may involve adding, replacing or deleting some sentences.

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## Materials and& Methods

1.

2.1.1) Turner A, Scalise S, Ralph Green, R, et al. (2013). Haemoglobin electrophoresis. In RMIT hHaematology pPractical mManual, page X-X. Melbourne, Australia: RMIT; 2013.

3.2.2) Ssickling test.

4.3.3) Bblood film

## Results:

**Table 1. Aanalysesis of the laboratory findings**

| Parameter | Result | Ref. range | N or ↑ or ↓ |
|---|---|---|---|
| RBCs | 2.54 x 1012/L | 4.1–5.5 x X1012/L | ↓ |
| Hb | 89 g/L | 105–140 g/L | ↓ |
| Hct | 0.23 | 0e.36–0.44 L/L | ↓ |
| MCV | 90.5 fL | 73–89 fL | N |
| MCH | 35 pPg | 27–32 pg | ↑ |
| MCHC | 387 g/L | 300–350 g/L | ↑ |
| Plt | 300 x 109/L | 150–400 x 109/L | N |
| WBCs | 11.9 x 109/L | 5–15 x 109/L | N |
| Ferritin | 25 ug/L | 15–50 ug/L | N |

**Commented [CE25]:** Please insert the missing page range here after a semi-colon. E.g. …2013. p. 1–15.

**Commented [CE26]:** Please check that you have satisfied the requirements of your course with what you have included in this section. Normally, you would explain that you have followed the procedure outlined in the manual and note any deviations from that procedure (e.g., errors, the need to retest, if something was done out of order or extra). If there are multiple kinds of sickling tests and blood films, you would also be specific about which ones you used.

**Commented [CE27]:** Please check my addition here, which was made for consistency. See also WBCs below.

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Note: RBCs = red blood cells; Hb = haemoglobin; Hc = haematocrit; MCV = mean corpuscular volume; MCH = mean cell haemoglobin; MCHC = mean corpuscular haemoglobin concentration; Plt = platelets; WBCs = white blood cells.

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**Table 2. Differential ~~WBC~~ white blood cell count**

| Parameter | Result | Ref. range |
|---|---|---|
| Neutrophil | 64 | 1.6–9 |
| Lymphocyte | 31 | 2–5 |
| Monocyte | 5 | 0.06–1 |
| Eosinophil | 1 | 0.1–1.4 |
| Basophil | 0 | 0.0–0.2 |
| NRBC | 2/100 WBC | 0 |

Note: WBC = white blood cells.

**Commented [CE29]:** Please add a definition for NRBC here as well. Note that each table should be self-contained, including the definition of abbreviations, because the tables could be reproduced on their own by others.

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**Table 3. Blood film comments**

| | |
|---|---|
| RBCs | Marked sSicklecel cells, moderate polychromatic cells, anisocytosis and a few nucleated RBCs |
| WBCs | Normal in number and morphology |
| Platelets | Normal in number and morphology |
| Sickling test | Positive |

Note: RBCs = red blood cells; WBCs = white blood cells.

## Discussion

The patient presented with a history of abdominal pain. The full blood examination revealed a decline in RBC count, Hb count and Hchaematocrit, but there was an increased in mean cell haemoglobin MCH and mean corpuscular haemoglobin concentration MCHClevels. However, MCVThe mean corpuscular volume level was normal. There were no changes in the morphology and quantity of the white blood cells (WBCs) and or platelets. Also s, and the serum ferritin appeared normal. On a peripheral blood smear, the Red blood cellsRBCs showed anisocytosis, marked sickle cells, moderate polychromatic cells and a few nucleated RBCs on peripheral blood smear. All tThese results all supported the provisional diagnosis of sickle-cell anaemia.

This diagnosis was confirmed by ttThe presence of turbid solution in the sickle-cell solubility test after adding a reducing agent confirm diagnosis. Also tThe alkaline electrophoresis



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Commented [CE30]: Please check that this abbreviation has been correctly expanded.

Note that if an abbreviation is only used once in the text, it is recommended to write the term in full only, rather than introducing the abbreviation unnecessarily.

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finding of ~~with the~~a pH of 8.6 also helped to~~s in~~ confirm~~ation of~~ the HbS band, with almost all of the ~~haemoglobin~~ Hb being ~~haemoglobin~~ HbS, and ~~the absence of~~ HbA being absent (the HbF band is difficult to see).

The percentage of HbS in cases of sickle-cell disease ~~is~~ ranges from 85-% to 100%, ~~-~~whereas HbF is usually not more than 15-% (with a range of ~~-~~2–~~-~~15%). Hb-F is found in high levels among the Arab-Indian haplotype and in h~~H~~ereditary ~~presestance~~persistence of ~~fetal~~foetal haemoglobin.~~–~~ HbA2 appears normal (at 2%) except in the Arab-Indian mutation (2~~1~~)~~-~~. The blood film test gives two ~~variety of~~ results: one ~~is~~ specific and the other ~~is~~ nonspecific. In ~~this~~ the case of this patient, the result is specific for sickle-cell disease, but ~~it~~ this ~~doesn't~~ does not exclude the presence of different concomitant ~~different~~ sickling disorders such as HbSC, HbSβ+ thalassemia and HbS β°thalassemia.

Sickle~~-~~-cell anaemia ~~-~~is an autosomal recessive inherited disease, with~~. So,~~ ~~-~~the patient having ~~inherits~~ inherited one copy of HbS from both parents.~~–~~ In this case, based on the result from Hb electrophoresis, the patient's parents ~~are~~ were heterozygous ~~based on Hb electrophoresis result~~ : t~~.~~hat is, they ~~are~~ had sickle-cell traits without clinical symptoms of anaemia. The incidence of disease is 25-% with each pregnancy~~-~~, heterozygosity will result 50-% of ~~them will have heterozygous~~the time, and ~~only 25 % will be normal~~the child will be born without any sickle-cell traits 25% of the time.

Co-inheritance of alpha thalassemia with sickle~~-~~-cell anaemia is found in some patients. ~~There is~~Approximately 30% of African~~o~~ ~~-~~Americans with HbS disease have the single alpha gene del~~e~~ation and 5% ~~with 2~~have a double gene~~s~~ del~~e~~ation (2~~1~~, 3)~~-~~. According to some studies, co inheritance of alpha thalassemia ~~thalasimia~~ with ~~SCD~~ sickle-cell disease results in an increased ~~the~~ chance of the survival of RBCs, with ~~helps reducing~~reduced ~~polymiraization~~polymerisation, less membrane damage and increased blood viscosity due to elevat~~ion in~~ed Hb concentration ~~.~~(4~~7~~). ~~that is~~ This leads to less severe haemolysis, reduced ~~occurance~~occurrence of symptoms and improved splenic function.

**Commented [CE31]:** Does this test measure the percentage of HbS? If so, this needs to be made clearer to relate the finding you report in the rest of the paragraph to the sentences at the beginning of this paragraph. For example, 'The percentage of HbS is measured by a blood film test, which gives two results: …'

**Commented [CE32]:** I have edited out the term Normal to describe people with no sickle-cell disease or recessive traits. Please check.

**Commented [CE33]:** Was this what happened in this case? If so, this needs to be clarified. Otherwise, this may not be relevant, or would be better placed in the introduction rather than in the discussion.

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Sickley, cell solubility tests, Hb electrophoresis or and chromatography aids in diagnosing sickle-cell disease. As tthe protein in Hb is negatively charged–, when testing byin the alkaline electrophoresis test, it migrates towards the anode (+).– In the sickle solubility test, the HbS are insoluble after addition of a reducing agent (e.g., sodium dithionite)., Hbhaemoglobin is released from the RBCs by a lysing agent, which becomes reduceds.– HbS crystallization crystallisation leading leads to the refraction of light and giving gives the a solution a turbid appearance. ButHowever, by this test, we cannot differentiate between homozygous and heterozygous. Tthe test might can also appear falsely negative due toin patients with a lower concentration of HbS. False negative test can occur or in patient has with low Hb, as well as in– neonates– less than six6 months old andor in post-transfusion cases. Old reagents also give a negative result. False positive results have been reported in Some cases like of lLeucocytosis and hyperproteinaemia, as and in cases of multiple myeloma was also reported to cause false positive results (3, 4 7). Similarly, HbC-Harlem, HbS-Travis and HbC-Zziguinchor also gives false positive results.

To exclude the other variants of HbS, like such as HbD and HbG, citrate agar, acid gel or isoelectric focusing– (IEF) are performed. The point mutation in a globin chain that can be tested by DNA-based tests. There isIn sickle-cell anaemia, there is an increase in lactase dehydrogenase and, unconjugated bilirubin, and a decrease of haptoglobin due to intravascular haemolysis in sickle cell anaemia. Identification of HbS/β°thalassemia from HbSS——— is carried out by HbA2 quantitation by using anion exchange column chromatography. HbF quantitation can be provide prognostic significance. The polymeriszation of HbS is interrupted by HbF, as it reduces the HbS quantity within the red blood cell RBCs (21, 3).

Hence, iIt can be concluded from the above case report that the severity of sickle-cell anaemia varies with different patients and much must be diagnosed in early childhood to improve the quality of life and chances of survival.



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**Commented [CE34]:** Are you saying that the lysing agent becomes reduced? Do you mean that the release of Hb reduces the RBCs? If so, consider rewriting this as 'which reduces the RBCs'.

**Commented [CE35]:** Homozygous and heterozygous what? Please clarify.

**Commented [CE36]:** Please check that this was in fact a typo.

**Commented [CE37]:** What is the relevance of the information in this paragraph to your case? This again may be better placed in the introduction, as the discussion should only include information directly relevant to discussing the findings for your case.

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**Commented [CE38]:** Note that only the results of tests you have performed and the implications of these should be included in the discussion. In this paragraph, it seems you are introducing a number of tests that you did not perform. Please check and either delete this paragraph or rewrite to clarify that you did perform these tests and what they showed about your case.

**Commented [CE39]:** Please revise this is possible. You do not appear to have performed any of these tests, and so you should not be introducing these in your discussion.

**Commented [CE40]:** Please check that this was what you meant to say.

**Commented [CE41]:** Should this be HbS?

**Commented [CE42]:** You do not appear to have shown this in the above discussion. In particular, you make no mention of quality of life or chances of survival in relation to early diagnosis. Please consider rewriting your conclusion to reflect what you have talked about in your discussion.

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## References

~~1 Bain JB. Sickle cell haemoglobin and its interactions with other variant Haemoglobins and with thalassemia. In Bain JB. Haemoglobinopathy Diagnosis. 2nd eds. UK: Blackwell Publishing Ltd, 2006; 139-189.~~


1. Frenette PS, Atweh GS. Sickle cell disease: old discoveries, new concepts, and future promise. The Journal of Clinical Investigation. 2007;117:850–858.
2. Bain JB. Sickle cell haemoglobin and its interactions with other variant haemoglobins and with thalassemias. In: Bain JB, editor. Haemoglobinopathy diagnosis. 2nd ed. Oxford: Blackwell; 2006. p. 139–189.

~~2.~~3. ~~3~~ Thein SL. Abnormalities of the structure and synthesis of haemoglobin. In: Porwit A, McCullough J, Erber WN, editors. Blood and bBone mMarrow ppathology. 2nd eds. USAPhiladelphia: Elsevier limited, 2011. p.; 131–155.

~~3.~~ Wild BJ, Bain BJ. Investigation of abnormal haemoglobins and thalassaemia. In: Bain BJ, Bates I, Laffan MA, Lewis SM. Dacie and Lewis practical haematology. 11th ed. Philadelphia: Elsevier; 2011. p. 301–332.

4.

~~4.~~5. ~~4~~ Roadak BF, Fritsma GA, Doig K. ~~HEMATOLOGY~~Hematology: cClinical pPrinciples and aApplications. 3rd rd ed. St. Louis, (MO) : ~~SAUNDERS~~ Saunders ~~ELSEVIER~~ Elsevier;; 2007.


**Commented [CE43]:** Please note that I have edited this reference list according to your university's Vancouver guidelines, as requested.

**Commented [CE44]:** I have reordered your references according to the order in which they were cited in your text. Originally, source 2 was cited first (this was changed to 1) and there was no 4, 5 or 6 (so, 7 was changed to 4).

Please check these changes carefully to ensure that each citation in your text is pointing to the correct source.

**Commented [CE45]:** Journal titles must be abbreviated in Vancouver-style referencing. I have revised this for you.

**Commented [CE46]:** Please avoid using superscript for ordinal numbers in the reference list.

**Commented [CE47]:** Please note that I checked these details for you on the Elsevier website.

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**Commented [CE48]:** Note that only 4 sources are used in the text. There is no number 5. Please check the correction of the first family name, which was based on Google Books.

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## Abstract

A seven-year-old boy presented complaining of abdominal pain. The given case report illustrates a case of anaemia that, after investigation, was found to be a case of sickle-cell anaemia. Many investigations of blood were undertaken, including a complete blood count, haemoglobin electrophoresis test and blood film examination. These initial investigations showed a decline in haemoglobin count, red blood cell (RBC) count and haematocrit, and an increase in mean cell haemoglobin and mean corpuscular haemoglobin concentration levels. Mean corpuscular volume was in the normal range. In addition to appearing fewer, the RBCs exhibited anisochromasia, and they had an abnormal sickle-shaped and boat-shaped appearance, with polychromasia. The first diagnosis was of anaemia, but the differential diagnosis also included sickle-cell/β° thalassemia and sickle-cell/haemoglobin C disease. In this case history, clinical examination and the case presentation delivered a great deal of knowledge for diagnosing the disease. Upon detailed investigation, a strongly positive result was found in the sickle solubility and sickling tests. A strong haemoglobin S was also found using alkaline electrophoresis, which confirmed sickle-cell disease.


## Introduction

The clinical history is helpful in most cases of haemoglobinopathy; it is a very useful tool in correct diagnosis. Knowing the patient's family history and ethnicity is also essential. Preliminary haematological tests, such as approximating the concentration of haemoglobin (Hb) and red blood cells (RBCs), also play a crucial role. Similarly, a comprehensive clinical examination of properly stained peripheral blood smears provides necessary knowledge for diagnosis. Other diagnostic tests, such as quantitation of HbA2 and HbF, the sickle solubility test, Hb electrophoresis and high-performance liquid chromatography, must also be conducted. Some other tests for determining the levels of ferritin in blood, total iron binding capacity and iron levels are also important to supplement the iron deficiency.

Sickle-cell anaemia manifests in childhood and is a disease that can easily be diagnosed in its early stages. The disease process begins with severe anaemia exhibiting some clinicopathological features and results in obstructed vessels due to the sickle-shaped RBCs causing tissue infarction. Vaso-occlusion occurs as a result of leukocyte recruitment and the coagulatory and inflammatory mediators of the sickle cells (1). Extravascular haemolysis is the outcome of chronic haemolysis (2).

The polymerisation of the sickle-shaped haemoglobin (HbS) due to the mutant Hb formed by the substitution of glutamic acid by valine in position six of the beta chain is the key pathophysiology involved in the disease process. This abnormal Hb becomes poorly soluble in the deoxygenated state and undergoes polymerisation under various conditions, including the concentration of HbS, temperature, hypoxia acidosis and factors such as 2,3-diphosphoglycerate concentration (2,3-DPG). The aggregation of HbS leads the RBCs to become rigid and less deformable. The repeat cycle of oxygenation, polymerisation and sickling disrupts cationic haemostasis, resulting in loss of water, $Ca^{2+}$ and $K^{+}$ from the cells. The living capacity of erythrocytes is based on the quantity of HbF and level of membrane damage. Sickle-shaped cells have a shorter lifespan, at around 20 days compared to 120 days for normal RBCs. The anaemia in sickle-cell disease is haemolytic anaemia. Patients with homozygous HbS exhibit the abnormal synthesis of beta chains. There is a lack of HbA, a different percentage of HbF, a small percentage of HbA2 (2, 3) and a small proportion of HbS.

The constriction of vessels, a very common symptom in patients with sickle-cell anaemia, causes an acute painful episode. The painful episode is more frequent when patients have low levels of HbF and alpha thalassemia with higher baseline Hb levels. Painful episodes are a common finding in young adults, but are less prevalent in elderly patients. In most cases, there is no identifiable cause, but pain episodes may be triggered by a cold, infection, menses, dehydration or stress. In young patients, pain episodes appear as dactylitis or hand-foot syndrome, in which there is swelling of the dorsal surface of the hands and feet. This swelling subsides within one to two weeks. Radiography reveals thinning of the cortex and some degenerative changes in the affected bones. In older children and adults, the most common sites of pain are the chest, abdomen, back and extremities.

Some laboratory tests to detect the presence of sickle cells were performed on blood film. Hb electrophoresis is a powerful tool for determining the sickling of Hb, to distinguish between the heterozygous state and other variants of the Hb band. Almost all the Hb is HbS and HbA is absent (the HbF band is difficult to see).

## Materials and Methods

1. Turner A, Scalise S, Green, R, et al. Haemoglobin electrophoresis. In RMIT haematology practical manual. Melbourne, Australia: RMIT; 2013.
2. Sickling test.
3. Blood film.

## Results

**Table 1. Analysis of the laboratory findings**

| Parameter | Result | Ref. range | N or ↑ or ↓ |
|---|---|---|---|
| RBCs | 2.54 x 1012/L | 4.1–5.5 x 1012/L | ↓ |
| Hb | 89 g/L | 105–140 g/L | ↓ |
| Hc | 0.23 | 0.36–0.44 L/L | ↓ |
| MCV | 90.5 fL | 73–89 fL | N |
| MCH | 35 pg | 27–32 pg | ↑ |
| MCHC | 387 g/L | 300–350 g/L | ↑ |
| Plt | 300 x 109/L | 150–400 x 109/L | N |
| WBCs | 11.9 x 109/L | 5–15 x 109/L | N |
| Ferritin | 25 ug/L | 15–50 ug/L | N |

Note: RBCs = red blood cells; Hb = haemoglobin; Hc = haematocrit; MCV = mean corpuscular volume; MCH = mean cell haemoglobin; MCHC = mean corpuscular haemoglobin concentration; Plt = platelets; WBCs = white blood cells.

**Table 2. Differential white blood cell count**

| Parameter | Result | Ref. range |
|---|---|---|
| Neutrophil | 64 | 1.6–9 |
| Lymphocyte | 31 | 2–5 |
| Monocyte | 5 | 0.06–1 |
| Eosinophil | 1 | 0.1–1.4 |
| Basophil | 0 | 0.0–0.2 |
| NRBC | 2/100 WBC | 0 |

Note: WBC = white blood cells.

**Table 3. Blood film comments**

| | |
|---|---|
| RBCs | Marked sickle cells, moderate polychromatic cells, anisocytosis and a few nucleated RBCs |
| WBCs | Normal in number and morphology |
| Platelets | Normal in number and morphology |
| Sickling test | Positive |

Note: RBCs = red blood cells; WBCs = white blood cells.

## Discussion

The patient presented with a history of abdominal pain. The full blood examination revealed a decline in RBC count, Hb count and haematocrit, but there was an increase in mean cell haemoglobin and mean corpuscular haemoglobin concentration levels. The mean corpuscular volume level was normal. There were no changes in the morphology and quantity of the white blood cells or platelets, and the serum ferritin appeared normal. On a peripheral blood smear, the RBCs showed anisocytosis, marked sickle cells, moderate polychromatic cells and a few nucleated RBCs. These results all supported the provisional diagnosis of sickle-cell anaemia.

This diagnosis was confirmed by the presence of turbid solution in the sickle-cell solubility test after adding a reducing agent. The alkaline electrophoresis finding of a pH of 8.6 also helped to confirm the HbS band, with almost all of the Hb being HbS, and HbA being absent (the HbF band is difficult to see).

The percentage of HbS in cases of sickle-cell disease ranges from 85% to 100%, whereas HbF is usually not more than 15% (with a range of 2–15%). HbF is found in high levels among the Arab-Indian haplotype and in hereditary persistence of foetal haemoglobin. HbA2 appears normal (at 2%) except in the Arab-Indian mutation (2). The blood film test gives two results: one specific and the other nonspecific. In the case of this patient, the result is specific for sickle-cell disease, but this does not exclude the presence of different concomitant sickling disorders such as HbSC, HbSβ+ thalassemia and HbS β°thalassemia.

Sickle-cell anaemia is an autosomal recessive inherited disease, with the patient having inherited one copy of HbS from both parents. In this case, based on the result from Hb electrophoresis, the patient's parents were heterozygous: that is, they had sickle-cell traits

without clinical symptoms of anaemia. The incidence of disease is 25% with each pregnancy, heterozygosity will result 50% of the time, and the child will be born without any sickle-cell traits 25% of the time.

Coinheritance of alpha thalassemia with sickle-cell anaemia is found in some patients. Approximately 30% of African Americans with HbS disease have the single alpha gene deletion and 5% have a double gene deletion (2, 3). According to some studies, coinheritance of alpha thalassemia with sickle-cell disease results in an increased chance of the survival of RBCs, with reduced polymerisation, less membrane damage and increased blood viscosity due to elevated Hb concentration (4). This leads to less severe haemolysis, reduced occurrence of symptoms and improved splenic function.

Sickle-cell solubility tests, Hb electrophoresis and chromatography aid in diagnosing sickle-cell disease. As the protein in Hb is negatively charged, in the alkaline electrophoresis test, it migrates towards the anode (+). In the sickle solubility test, the HbS are insoluble after addition of a reducing agent (e.g., sodium dithionite). Hb is released from the RBCs by a lysing agent, which becomes reduced. HbS crystallisation leads to the refraction of light and gives the solution a turbid appearance. However, this test cannot differentiate between homozygous and heterozygous. The test can also appear falsely negative in patients with a lower concentration of HbS or with low Hb, as well as in neonates less than six months old or in post-transfusion cases. Old reagents also give a negative result. False positive results have been reported in cases of leucocytosis and hyperproteinaemia, and in cases of multiple myeloma (3, 4). Similarly, HbC-Harlem, HbS-Travis and HbC-Ziguinchor give false positive results.

To exclude the other variants of HbS, such as HbD and HbG, citrate agar, acid gel or isoelectric focusing are performed. The point mutation in a globin chain can be tested by DNA-based tests. In sickle-cell anaemia, there is an increase in lactase dehydrogenase and unconjugated bilirubin, and a decrease of haptoglobin due to intravascular haemolysis. Identification of HbS/β°thalassemia from HbSS is carried out by HbA2 quantitation using anion exchange column chromatography. HbF quantitation can provide prognostic significance. The polymerisation of HbS is interrupted by HbF, as it reduces the HbS quantity within RBCs (2, 3).

It can be concluded from the above case report that the severity of sickle-cell anaemia varies with different patients and must be diagnosed in early childhood to improve the quality of life and chances of survival.

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