International Journal of Applied Engineering Research ISSN 0973-4562 Volume 15, Number 7 (2020) pp. 721-729
© Research India Publications. http://www.ripublication.com

Quality Improvement of Microscopic Images for the Detection of Megaloblasticanemia

Hariharan S.

Professor, Department of Biomedical Engineering,
TKM institute of Technology, Kollam, Kerala, India.

Abstract

Megaloblasticanemia occurs because of the deficiency in vitamin B_{12}, folate or defects due to DNA synthesis. The method of placing the blood smear in microscope and counting manually the number of cells is a difficult and boring process and put more stress for the medical laboratory technicians. The computer aided systems can provide more accurate results in small amount of time. In the present work analysis of megaloblasticanemia images are considered and used them for the detection and confirmation of this rare disease. Megaloblasticanemia images are collected from various sources and processed in computer to get fast and reliable diagnostic results by improving the quality of the image.

Keywords: Anemia, Megaloblasticanemia, blood smear image analysis, Computer aided diseases diagnosis.

INTRODUCTION

Biomedical images can broadly be classified into microscopic and macroscopic. The microscopic image analysis is found to be extremely important in the diagnosis of some rare diseases and microscopically small objects like cells or tissues. Neutrophil segmentation is found to be one of the major criteria in the diagnosis of megaloblasticanemia. These segmented neutrophils usually seen as a single structure. By finding the number of lobles in neutrophils it is possible to do the diagnosis of this disease condition.

Many specific studies have been conducted in the case of megaloblasticanemia based on certain criteria but a reliable and faster method is very much essential for the detection of this blood related disease. In an article on stroke as an initial themine –responsive megaloblasticanemia Madaan.P, Jauhari.P and Gulathi.S have discussed some aspects of megaloblasticanemia [1]. One of the important case study conducted by Sarode.AM, Yeolekar.ME and Harel.S reveals the presence of megaloblasticanemia in chronic alcoholics [2].In one of the interesting article on megaloblasticanemia AnisHariz and Priyanka T Bhattacharya, examined both megalloblastic and non-megaloblasticanemias. They reveal that the primary work of physicians is to check whether hemolyticanemia or acute hemerage are the main two conditions. The presence of megaloblasticanemia may be due to alcoholism, hypothyroidism, liver dysfunction or drugs [3].

Krishna Prakasha and Vasundara Acharya worked on anemia images and developed a computer aided technique for separating red blood cells, classify them and diagnose sickle cell anemia. This work defines a systematic approach for classifying the red blood cells with and without pallor [4]. In a review on detection of sickle cell anemia in red blood cells , Amitkumar Biswas indicated that an ideal computer aided technique for distinguishing sickle cell anemia is not existing but fractal dimension may be one of the best choice[5].A paper on megaloblasticanemia has been published by S.Sreekanth. He has conducted a hospital based retrospective and prospective study for a period of one year. He has analyzed 21 cases in the age group of 2 months to 15 years. Megaloblasticanemia was observed in all cases[6].For the accurate diagnosis of blood related diseases such as sickle cell anemia, pancytopenia, megaloblasticanemia etc… examination of bone marrow is very much essential. E.P Weinzierl and D.A Arbert have conducted a study on pancytopenia and brought out the causes of it in their work on review and overview of pancytopenia[7].

In their work on megaloblasticanemia, Asleena, J.J.Maaz and S.H.Yale points out that megaloblasticanemia is one of the causes of macrocytosis. They have also suggested methodologies for the treatment of vitamin B12 and folate deficiencies[8].In this study mean corpuscular volume is calculated and based on that megaloblasticanemia and other causes of macrocytosis have been investigated. They observed that the common pathological causes of macrocytosis are due to vitaminB12 deficiency, folate deficiency, hypothyroidism, alcoholism non alchoholics and alcoholic liver diseases etc…. .They conducted experiments on blood smears and observed the mean corpuscular volume value as 134 fl in the case of vitamin B_{12} deficiency patients and a value of 114 fl in patients with liver diseases. Studies on megaloblasticanemia and other related diseases based on mean corpuscular volume values have also been reported by many researchers[1,6, 9,12,49,23,5].

A Brown, N.Tendon, S.R Mehdi and Z.Siddiqi have conducted a biological studies on megaloblasticanemia patients. The aim of their study was to evaluate the hematological presentation of patients of megaloblasticanemia associated with deficiency of vitamin B12 and folic acid. In their observation among the 90 patients examined most of them had vitamin B_{12} deficiency. Their conclusion was that there is a significant difference in the clinical presentation of patients of Megaloblasticanemia with B12 and folic acid deficiency. Another conclusion was that there is no significance in the hematological parameters of megaloblasticanemia[9].The authors recommend that both clinical and haematological parameters shold be assessed properly for distinguishing folic acid deficiency patients and vitamin B_{12} deficiency

patients[[16,13,18,10]

Megaloblasticanemia has emerged as a cause of morbidity and mortality in tropical countries like Africa, America and India. Hindu communities are affected by this disease mainly. Vegetarians are more susceptible to megaloblasticanemia. Vegetarian diet protect folic acid deficiency but not cobalamin deficiency. The authors concludes that proper diagnosis is essential for the use of computer aided diagnostic techniques to find megaloblasticanemia[10]

One of the interesting aspect of megaloblasticanemia is due to vegetarianism. This phenomenon has been reported by H.S.Bawwaskar, P.H.Bawaskar, P.B.Prekh etal[11]. Oral vitamin B_{12} replacement at 100 μg daily is an adequate alternative to vitamin B_{12} injections. Deficiency of vitamin B_{12} , vitamin D, folic acid or iron deficiency are common among Indians. The reasons for this are due to diet, life style and social & cultural issues. The systems were modified by the underlying disorder causing its deficiency. B_{12} deficiency is a common disorder in India. It's recognition is missed or delayed because their manifestations are diverse in nature. It will affect all organs and systems. It is found that most of the time the lab estimations are unreliable.

In their work on determination of nutritional anemia in adolescents Deena Thoms, J.Chand , S.Sharma A Jain and H.K.Pande describe various anemias and their characteristics[12]. The objective of their study was to associate the severity of nutritional anemia with serum levels of ferritin vitamin B12 and folate to determine demographic socioeconomic and nutritional correlates for nutritional anemia in adolescents. The results of their study illustrated that iron folate and B12 deficiency was present in 30.5 %, 79.5%, and 50% of adolescents respectively. They also observed that statistically significant association was present between severity of anemia and serum vitamin B_{12} levels, iron intake and vitamin B_{12} intake and vegetarian diet. Their conclusion was that folate and B_{12} deficiency are more common in iron deficiency in anemic adolescents.

Megloblasticanemia patients can be treated with vitamin B12 supplements statins and antiplatelets. They can be treated with protein rich diet including fruit and vegetables.

An effort for the identification of megaloblasticanemia cells through the use of image processing techniques was put forward by Asaad Babker and V Lyashenko[13] . Their aim was to show the possibility of using image processing techniques to identify megaloblasticanemia cells. Apart from determining image processing operations, they have also used wavelet transforms for analysis of Megaloblasticanemia images. Wavelet transforms have also been used by other researchers **[7,12,8]** for the processing of megaloblasticanemia images. However a perfect image processing system for the detection of megaloblasticanemia is not yet developed.

Various aspect of megaloblasticanemia and other blood related diseases have been discussed by H.P.Pandey and A.Patel in their work on clinical profile of patient of megaloblasticanemia [14]. Their aim was to find the objective pattern with its clinical profile of megaloblasticanemia. This work observed that megaloblasticanemia was the highest in patients between the age group of 40 and 49. In a work on vitamin B_{12} and folic acid related megaloblasticanemia.I.Karakoyun, C.Duman and F.D Arslan studied some aspect of megaloblasticanemia [15]. They have tried to investigate the effect of megaloblsticanemia due to vitamin B_{12} or folic acid deficiency on the levels of tumour markers. The study concludes that megloblasticanemia results in ineffective erythropoiesis.

B.K.Singh and Indalkumar conducted a study on pancytopenia which is a common presenting feature of megloblasticanemia[16]. The study specifically states that, the most common cause of pancytopenia is the megloblasticanemia. The conclusions of this study was that megloblasticanemia and aplastic anemia were amongst the common etiological diagnosis of pancytopenia and severe anemia was common in megloblasticanemia. S.N.Anjum and A.M.Naikwadi conducted a study on pancytopenia. [17].Shah.Petal … worked on the diagnosis of bone marrow aspiration [**18]**. Pancytopenia study was also conducted by B.N.Gayatri and K.S.Rao in their work on clinical haematology study[**19]**

A case study had been conducted by P.Diaz ,M.A.VieiraA.Carneiro and N.Fernandes highlighting the importance of differential diagnosis of pancyopenia. A rare disease called Systemic lupuserythematosis (SLE) is a chronic disease affecting multiple organs. This is usually associated with pancytopenia[20]. An interesting review written byD..SochaS.I.Desouza, Aron Flagg etal... brings out important aspects of megaloblasticanemia. They made two case studies one on woman with anemia and the other is an old man with mylodisplstic syndrome. They specifically says that, the most common cause of megaloblastic anemia are deficiencies of B9 (folate) or vitmin B12(cobalamine)[21]. In this work on vitamin B12 deficiency, Robert. C presents megaloblasticanemia along with peripheral neuropathy and neuropsychiatric complaints. He also points out that once the diagnosis of megaloblasticanemia is completed the causes of B12 deficiency should be searched and oral vitamin B12 therapy may be started.[22]

An automatic image segmentation technique for counting blood cell nuclei in megaloblasticanemia had been proposed by H.M.Chen Y.T. Tsao and S.C. Tasi[23]. Clinical profile of megloblasticanemia has been studied by BR. Pokharel,P. Pant, R.GURUNG, R.Koju TRS Bedi[24]. Their work was the outcome of 6 years treatment experience in Kthmandu university hospital, Nepal India[25]. Hariz.A and Bhatacharya PT published a book chapter on megloblasticanemia which they illustrate that this rare disease is due to hypovitaminosis specifically cobalamine and folate which are necessary for the synthesis of DNA.[26]. Megloblasticanemia is mainly due to the presence of bone marrow of large red blood cells which are called megaloblasts. One of the main causes of megaloblasticanemia is ineffective red blood cell production. The common cause of megloblasticanemia is deficiency of cobalamine and folate[27]. VasundaraAcharya and Preethamkumar developed a method for classification of red blood cells or identification of blood related diseases [28]. There are many research articles describing

megaloblasticanemia and other blood related diseases [29-44].

MATERIALS AND METHOD

A data base has been created collecting images from various sources. They are verified by an experienced medical professional and made sure that these patients are affected with megaloblasticanemia. If the neutrophils are segmented into three or more lobes we can make sure that these people are affected with megaloblasticanemia. Image processing operations are performed on these images to check whether some clinical information can be drawn from the processed images. The ultimate aim of this work is to implement simple and reliable image processing operations for the diagnosis of megaloblastic anaemia. Here different types of image processing operations are performed for image quality improvement. Images are filtered using median filter. The reason for using median filter is that it modifies the image in such a way that noise will be eliminated to a considerable extent and will not alter the information content extensively.

Edge is considered to be one of the important aspect of image processing where the human visual system concentrate more for deriving information and decision making process to segment one region to distinguish from other similar regions within an image. It is at this point where maximum intensity variation occurs. Edge detection has been performed to make the object edges more clear and legible. By observing the red blood cells it is possible to check whether circles (which are the edges of the RBC's) and cell has been enlarged to some extent. One of the important aspect of megaloblastic anemia is that the RBC's will be enlarged and then neutrophil segmentation takes place. It is possible to do diagnosis by observing the number of lobes in the neutrophil.

Another set of image processing operation performed on megaloblastic anemia images is the segmentation. Segmentation of megaloblastic anemia images of neutrophils which are divided into different lobes can be observed and counted. It is clear and easy to understand for human beings to decide how many lobes are there in the segmented neutrophils in the microscopic images.

One of the aims of this work is to develop a simple image processing system which is easy to implement in clinical environment. The theory used for producing the output images is also simple and easy to use. Matlab software has been used here for the implementation of these standard image processing techniques. The readers can refer(Ref:44)for the theoretical aspects of these system which are widely used by the academic community throughout the world.

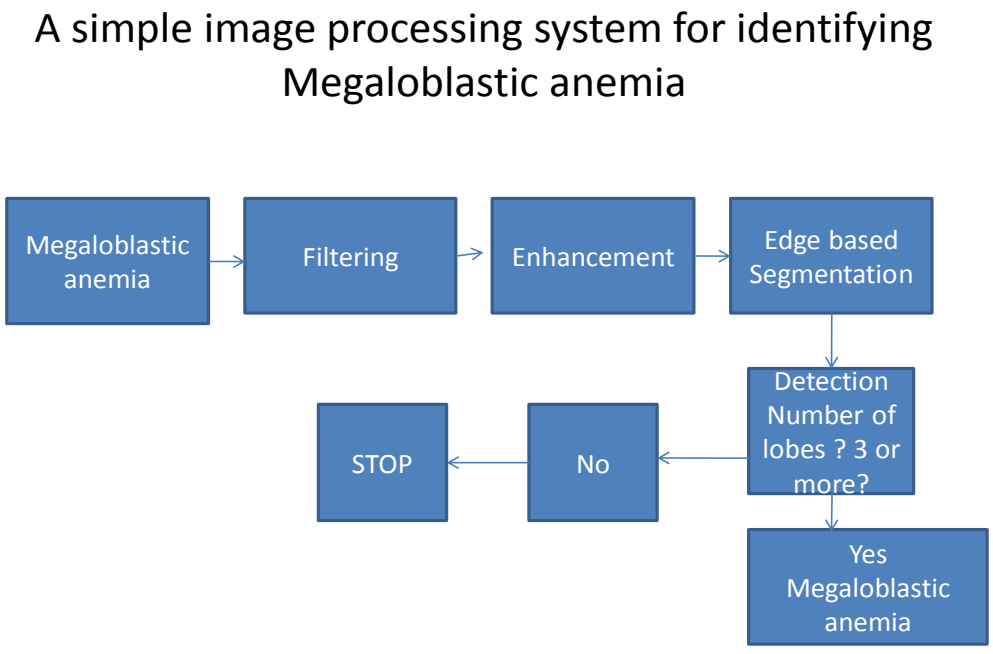


Fig 1 Image processing system for megaloblastic anemia

RESULTS AND DISCUSSIONS

Getting good quality images is the dream of every physicians, scientists and biomedical engineers. For implementing any system in clinical field, it is necessary to use most simple image processing techniques than complex and complicated algorithms. With this point in mind few image processing operations are implemented on a megaloblastic anemia image and obtained better quality images as illustrated in figures shown below.

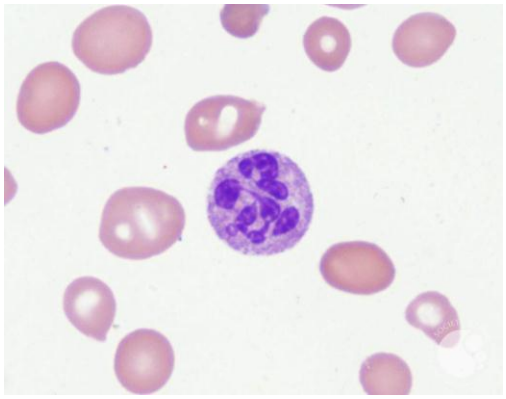


Fig 2.a: Megaloblasticanemia (sample-1)

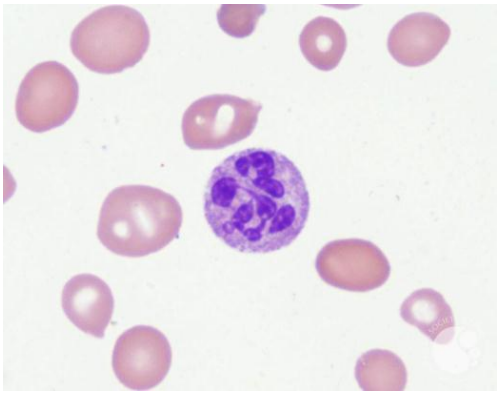


Fig 2.b: Contrast enhanced version of Fig 2.b

In Fig2.a, a neutrophil segmented image is shown. It is possible to see that it has been segmented into eight parts. For confirmation of megaloblastic anemia, the number of lobes should be three or more. So this is a sure case of megaloblastic anemia. We can also count the number of lobes automatically. Many scientists have already developed the methods for lobe counting in blood cells. The same strategy can be implemented here also. But it is not significant in this work because the division is clear while looking through the human visual system. It is possible to do disease diagnosis accurately without any processing operation. In Fig2.b contrast stretching has been used for enhancing the image. Fig 2.b is much more better than 2.a and its contrast has been increased.

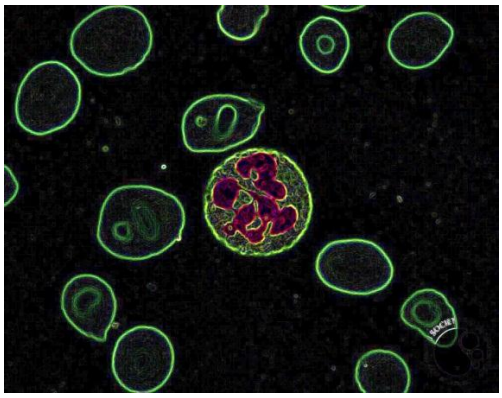


Fig 2.c: Edge detected

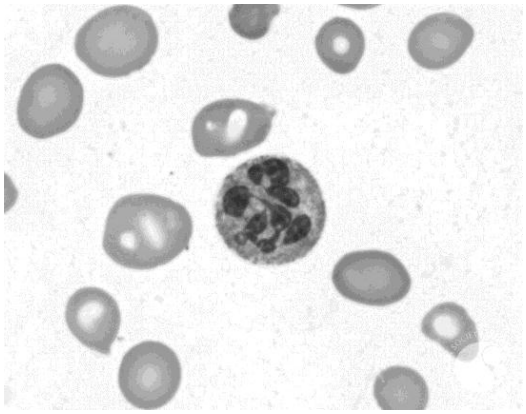


Fig2.d: grey scaled image of Fig2.a

In Fig2.c edges of the cells are highlighted and it is possible to see any abnormal enlargement of the cell is present. It is possible to observe from this figure that one cell is enlarged and even the edges of the nuclear lobes can be counted. Image is then grey scaled as shown in Fig 2.d for further processing.

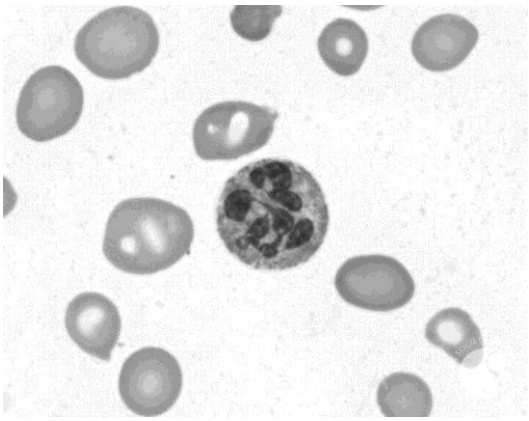


Fig 2.e : Enhanced

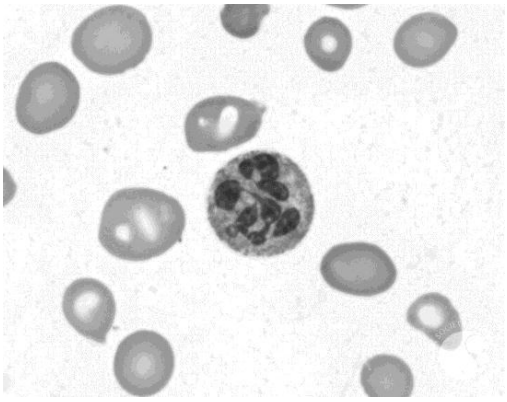


Fig2.f: Enhanced more

In Fig 2e image contrast has been increased by using unsharp-masking. In Fig2.f The image contrast is further increased with contrast stretching.

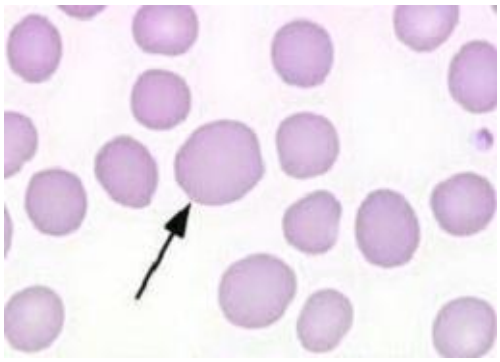


Fig3.a.: Megaloblasticanemia(sample-2)

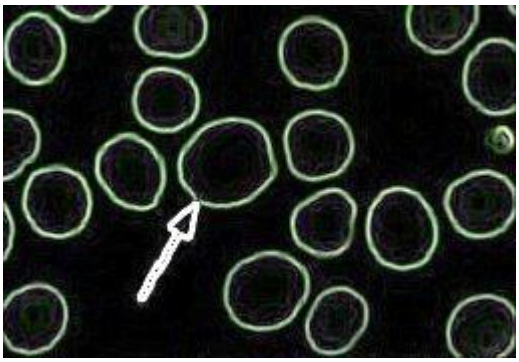


Fig 3.a-1: Edge detected version Fig3.a

The results obtained in this image analysis work of Megaloblastic anemia are summarized below. Fig3.a, 3.b 3.c and 3.d are the blood smear images of Megaloblastic anemia patients. One of the aims of this work is to check whether any clinical information can be extracted using simple image processing techniques. Before actually starting the neutrophil segmentation first of all RBC are enlarged. Early detection of Megaloblastic anemia is possible because of the enlargement of RBC's. In Fig.3.a one of the cell is enlarged(shown by arrow). By detecting the edges it is possible to clearly identify the enlarged cells from the normal ones which are similar in size and shape. This can be observed from Fig.3a-1.It is possible to observe that there are so many information inside the cells which are not clearly visible but its presence can be identified**.**

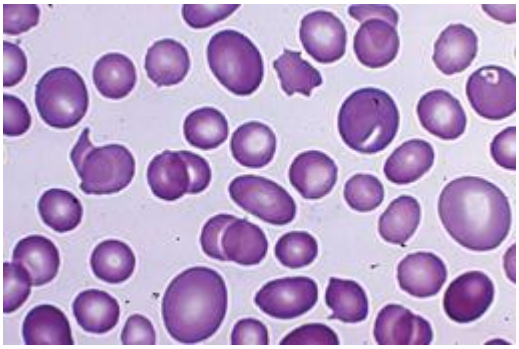


Fig 3.b: Megaloblasticanemia (sample-3)

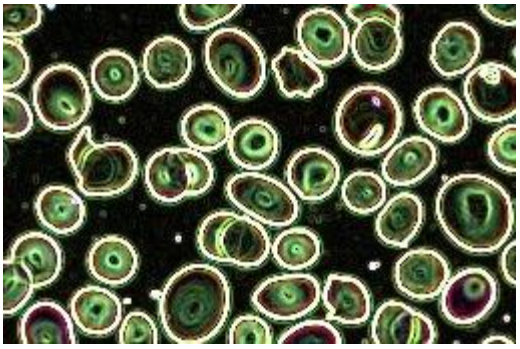


Fig3.b-1: Edge detection of Fig3.b

Fig 3.c and 3.d re two megloblsticaneimia images. In 3.c the information contents in inside of the cell is not clear. Whereas after edge detection it is possible to see many information inside. It can be seen from Fig 3.c(1).Number of neutrophils inside cells can be easily seen, enlarged cells, touching and non touching cells cell division all these information is revealed as in Fig 3.c(1). Other components inside the extracellular fluid is also possible to see in the edge processed image where as they are not prominent in the input image.

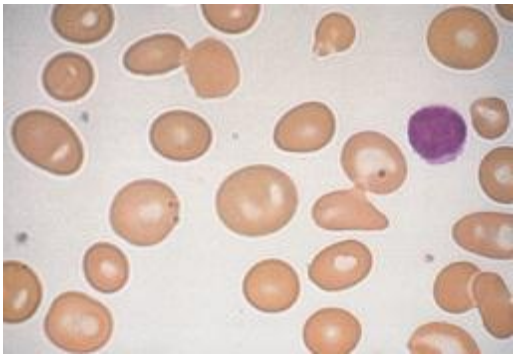
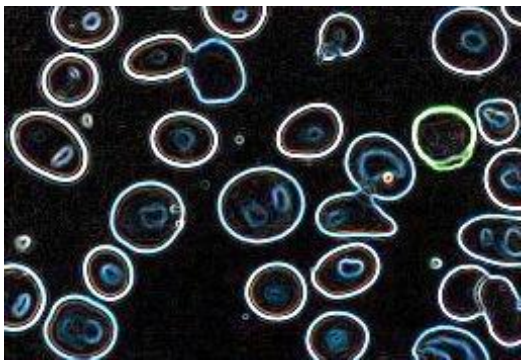


Fig3.c: Megaloblasticanemia (sample-4)



3.c-1: Edge detection of Fig3.c

In Fig3.d one enlarged cell and normal cells can be visualized. In the edge detected version it is possible to see several information inside the cells including number of lobes. It can be observed that in the input image the intensity of the image inside the cell is almost uniform and looks like there is not much important components. But in the output image 3.d-1 we

can see that many information is hidden which requires further analysis to retrieve the information content.

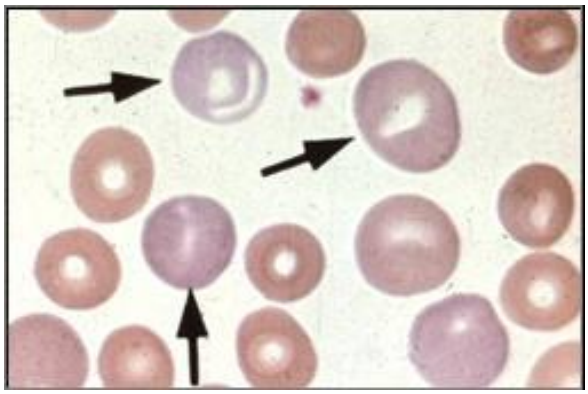


Fig 3. Dmegaloblasticanemia (sample-5)

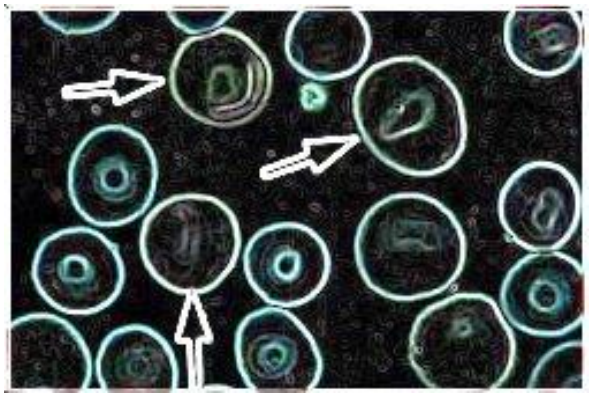


Fig3.d-1: Edge detection of Fig3.d

Fig4.1 – is the original image used for implementing image processing operations which is a sure case of megaloblasticanemia. Fig 4.2 is obtained after contrast stretching which is one of the most simple and elementary image processing operation. It is possible to see much better quality image than the input image.

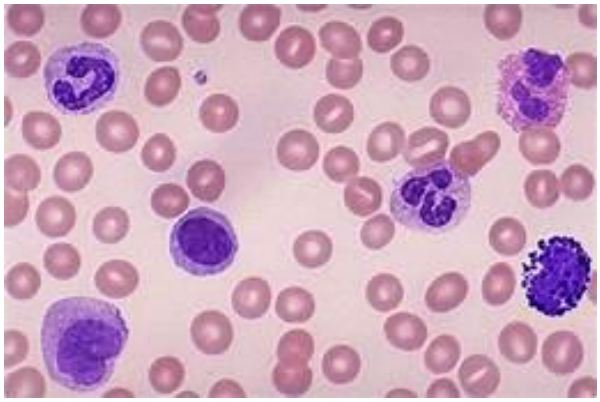


Fig4.1: Megaloblasticanemia(sample-6)

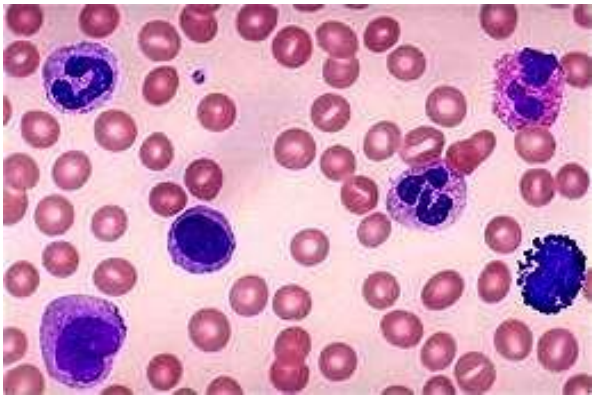


Fig4.2: Contrast stretching of Fig4.1

In Fig.4.3 edges are highlighted which reveal clues for medical professionals. However they need further advanced operations for getting clinical information. In Fig4.4 one of the classical image processing operation is performed which is nothing but modified histogram equalisation technique, we call it clipped histogram equalisation.

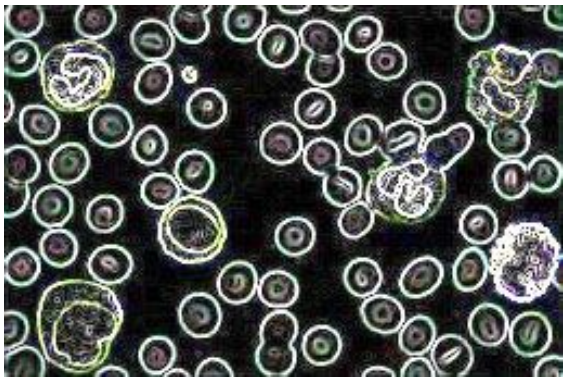


Fig 4.3: Edge detected

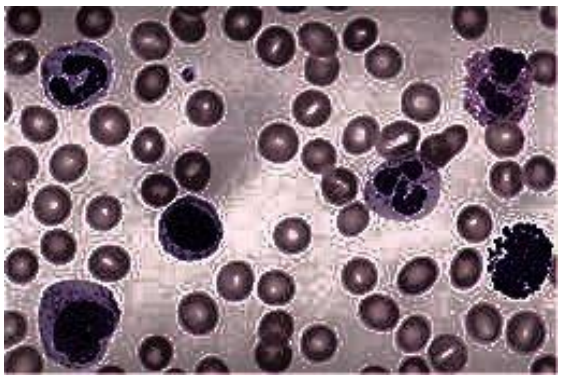


Fig 4.4: Clipped histogram equalisation

The main disadvantage of classical histogram equalisation is that it over enhance the image. But it is very much suitable for low contrast images, like x-ray images.

CONCLUSIONS

Megaloblasticanemia is a rare, blood related disease, the diagnosis of which can be done by the analysis of blood smear images. The disease affected blood samples are collected from medical colleges inKerala, India and these are used as input images for applying image processing operations. Images are enhanced using un-sharp masking and contrast stretching. For edge detection, canny edge detector is being used.A simple image processing system has been developed in this work which can identify megaloblastic anemia based on visual inspection along with the computer processing. All the output image obtained in this work are found to be much better utility than the input images of megaloblastic anemia. Hence disease diagnosis is much more easy and accurate for physicians and other medical professionals.

ACKNOWLEDGEMENTS

The author is grateful to the physicians who supplied the sufficient number of images for conducting this work. The author thank Dr.Ramachandran.K retired professor, Regional cancer centre, Trivandrum, Kerala, India, presently working in Indian institute of Diabetes, Kerala, South India for confirming the megaloblastic anemia images to create the data set.

REFERENCES

[1] Madaan.P, Jauhari.P and Gulathi.S, "Stroke as an initial thianmine responsive megaloblastic anemia", Ann Indian Acad neural February 2020 234(1): pp136-138.

[2] Sarode AM, Yeolekar ME, Harel S, " Megaloblastic anemia in chronic alcoholics: A case series" J Assoc Physicians India. 2020 Jan;68(1):98.

[3] AnisHariz, Priyanka T Bhattacharya, "Megaloblastic anemia", Treasure Island (FL): StatPearls Publishing; January 2020

[4] VasundharaAcharya and Krishna Prakasha, " Computer aided technique to separate the RBC, categorize them and diagnose sickle cell anemia*"*, Journal of engineering science and technology review, April 2019, 12 (2) pp 67-80

[5] AmitkumarBiswas, "Detection of sickle cell anemia in RBC: A Review" International journal of advances in Engineering sciences and applied mathematics, March 2015 2(1), pp45-48.

[6] S.Sreekanth," Megaloblastic anemia – a clinical spectrum and a hematological profile- the day to day public health problem " Medical journal of Dr5t.D.Y.Patil University, June 2016, vol-9, Issue-3, February 202:0,pp 307-310

[7] E.P.Weinzierl and Daniel A.Arbar, "The differential diagnosis and bone marrow evaluation of new onset pancytopenia", American journal of clinical pathology, Vol-139, Issue-1, January 2013, pp9-29

[8] Florence Aslinia, Joseph J. Mazza and Steven H. Yale,*"*Megaloblastic anemia and other causes of macrocytosis*"* Clinical medicine and research,*2006, Vol-4, No-3, pp236-241*

[9] Amreen Brown, Nishi Tandon, Syed Riaz Mehdi, ZebaSiddiqi, " Varied Clinico-haematological Presentation of Patients of MegaloblasticAnaemia with Deficiency of Vitamin B12 and Folic Acid in a Tertiary Care Center of Northern India", Journal of Clinical and Diagnostic Research. 2017 Dec, Vol-11(12): EC06-EC09,pp6-9

[10] PraphullDeepankar, RakeshRoshan, Harish kumarGuptas, GurdeepBuxi, *"* Relative prevalence of Vitamin-B12 and folic acid in Megaloblastic anemia and its clinical –etiological profile in a tertiary care center" International journal of scientific study, June 2018, vol-6, Issue-3 pp23-30.

[11] HimmatraoSalubaBawaskar, ParagHimmatraoBawaskar, PramodiniHimmatraoBawaskar, Punishi B Parekh , " Tropical megaloblastic anemia" The Lancet Vol-393 Issue10187,P2261 June 01 2019, DOI: https://doi.org/10.1016/s0140-6736(19)31123-7

[12] Deena Thomas, J.Chand, S.Sharma and A.Jain and H.K.Pemde, " Determinant of nutritional anemia*"* P.K.Sasidharan,"TY - CHAP, PY - 2006/01/01, SP - 319,EP - 330,SN - 9788180615856 T1-Chapter-13 Anaemia: Clinical Evaluation,DO - 10.5005/jp/books/10224_13

[13] AsadBabker, VyacheslavLyashenko, " Identification of megaloblastic anemia cells through the use of image processing techniques,*"* International journal of clinical and Biomedical research, 2018, Sumathi publications pp1-5

[14] H.P. Pandya and A.Patel "clinical profile and response in patients with megaloblasticanemia "International journal MdiclinePubli health, 2016 5(2) 304-306.

[15] InancKarakoyun, Can Duman, F.D.Arslan, Anil Baysoy, BanuIsbilenBasok "Vitamin B12 and folic acid associated megaloblastic anemia" March 11 2019

[16] B.K.Singh&Indalkumar, " Pancytopenia a common presenting feature of megaloblasticanemia"DOI:http://www.doi.org/10.3610 6/paripex.

[17] S.N.Anjum and A.M.Naikwadi, " Eitiloloy of pancytopenia and its bone marrow picture" International journal of contemporary medical research, Vol-2 Issue3 July 2019 pp45-48.

[18] Shah. P, etal " Bone marrow examination in case of pancytopenia, International journal of Research in medical science 2007, 5 , pp 1494-1498

[19] B.N GayatriKadamsathyanarayanarao, "Pancytopenia: A clinico hematological study, " Journal of laboratory physicians 2011, 3, pp15-20

[20] Diaz P, Abreu Vieira M, Carneiro A, Fernandes N. “” A case of pancytopenia with many possibile causes: how do you tell which is the right one? “ EJCRIM ,2019; 6 , European Journal of Case Reports in Internal Medicine : doi:10.12890/2019_001012.

[21] Priscila Diaz , Mariana Abreu Vieira, AntónioCarneiro, NatáliaFernandes, Department of Internal Medicine, Cascais Hospital, Cascais, Portugal, “A Case of Pancytopenia with Many Possible Causes: How Do You Tell Which is the Right One?” , European Journal of Case Reports in Internal Medicine © EFIM 20:DOI: 10.12890/2019_001012

[22] Daniel S. Socha, Robert J. Tomsich , Sherwin I. DeSouza, Aron Flagg, New Haven, MikkaelSekeres, OH Heesun J. Rogers, Robert J. Tomsich, Severe megaloblastic anemia: Vitamin deficiency and other causes Cleveland Clinic Journal of Medicine March 2020, 87 (3) 153-164; DOI: https://doi.org/10.3949/ccjm.87a.19072

[23] H.M.Chen Y.T. Tsao, S.C. Tasi*“* Automatic scheme for counting the cell nuclei with megaloblastic anemia” Journal of medical imaging and health informatics 6(1) 2017 p 102-107.

[24] BR. Pokharel,P. Pant, R.GURUNG, R.Koju TRS Bedi, “Study of clinical profile of megloblastic anemia :an experience of 6 years at kathmandu University Hospital”journal of College of medical sciences Nepal Vol. No.2 ,2011 Dhulikhel. http/doi.org/10.3126/cmsn.v71.2

[25] V.Lyashenko, A Babker “Using of color model and contrast variation in wavelet ideology for study megaloblastic anemia cells” Scientific research publishing **2017.**

[26] Hariz A, Bhatacharya. PT “Megaloblastic anemia” StatPearls Publishing LLC,Treasure island (FL) **2020** January.

[27] Soha DS DeSouza SI etal….. “Severe megaloblastic anemia deficiency and other causes” Cleve Clin J Med **2020** March 87(3) pp153-16

[28] Vasundaraacharya and preethamkumar, “Identification and red blood cell classification using computer aided system to diagnose blood disorders”2017 International Conference on Advances in Computing, Communications and Informatics (ICACCI) IEEE 13-16 Sept. 2017, Udupi, India, **DOI:** 10.1109/ICACCI.2017.8126155

[29] Ralph Green, “Vitamin B12 deficiency from the perspective of a practicing haematologist” vol. 129, Blood, May 2017 pp. 2603-2609

[30] Yoganathan Chidambaram, Anith Kumar Mambatta, Sujith K. Sivaraj, “Tropical sprue in megaloblastic anemia, International Journal of Research in Medical Sciences, September 2017, Vol 5, Issue 9, pp. 4133-4136

[31] SouryaAcharya, Samarth Shukla, AjinkyaJamthe, AnkitaTamhane, Sickle Cell Anemia with Megaloblastic Crisis: Deficiency or Demand, World Journal of Anemia, April-June 2017, pp.63-64

[32] Moghadam, S. and Ghorbani, M. A Case of Megaloblastic Anemia with `Thrombocytopenia.Open Journal of Obstetrics and Gynecology, Vol. 6, pp. 534-538, 2016

[33] SatadalBarik, “Megaloblastic Anemia: A Drug-Induced Disorder”, J Blood DisordTransfus, Volume 7, Issue 3, 1000359

[34] Yadav MK, Manoli NM, Madhunapantula SV, “Comparative assessment of vitamin B12 folic acid and homocystine levels in relation to P53 expression in megaloblastic anemia”PLoS One. 2016 Oct 25;11(10):e0164559. doi: 10.1371/journal.pone.0164559. eCollection 2016.PMID: 27780269

[35] Uma Khanduri, Archna Sharma, “Megaloblastic Anaemia: Prevalence And Causative Factors”, The National Medical Journal Of India vol. 20, no. 4, 2007, pp.172-175

[36] H.B. Castellanos-Sinco, C.O. Ramos-Pe˜nafiela, A. Santoyo-Sánchezc, J. Collazo-Jalomaa, C. Martínez-Murilloa, E. Monta˜no-Figueroaa, A. Sinco-Ángelesd, “Megaloblastic anaemia: Folic acid and vitamin B12 metabolism”, Rev Med Hosp Gen Méx. 2015; Vol. 78, No.3, pp.135-143

[37] Danielle R. Rusher and Roman Pawlak**, “** A Review of 89 Published Case Studies of Vitamin B12 Deficiency”, Journal of Human Nutrition & Food Science, 2013, Vol. 1, No.2

[38] AmieleenaChhabra, VipanChandar, Alpa Gupta, Harish Chandra, “Megaloblasticanaemia in hospitalised children”, Journal, Indian Academy of Clinical Medicine l Vol. 13, No. 3 l July-September, 2012, pp.195-197

[39] B.R. Pokharel, P. Pant, R. Gurung,, R. Koju, T.R.S. Bedi , R. Makaju, S. Sanjel, “Study of clinical profile of megaloblastic anemia: An experience of six year at Kathmandu University Hospital, Dhulikhe*l*”, Journal of College of Medical Sciences-Nepal,2011,Vol-7,No-2, pp. 41-44

[40] Jagdish Chandra, “Megaloblastic Anemia: Back in Focus”, Indian Journal of Pediatrics, Volume 77—July, 2010, pp.795-799

[41] Florence Aslinia, MD; Joseph J. Mazza, MD, MACP; and Steven H. Yale, MD, FACP, “Megaloblastic Anemia and Other Causes of Macrocytosis” , Clinical Medicine & Research, Volume 4, Number 3, pp.236-241

[42] ValentinaLabay, Tal Raz, Dana Baron, Hanna Mandel, Hawys Williams, Timothy Barrett, RaymondeSzargel, Louise McDonald, Adel Shalata, Kazuto Nosaka, Simon

Gregory & Nadine Cohen, *"*Mutations in SLC19A2 cause thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness*"*, Nature genetics , volume 22 , july 1999,pp.300-304

[43] L.S.P Davidson, Lond, " Refractory megaloblastic anemia", Blood journal, 1966 pp. 107-120

[44] Gonzalez RF, Wintz P. Digital image processing. 3. Addison-Wesley; 1992. [Google Scholar]