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Structural and textural classification of erythrocytes in anaemic cases: A scanning electron microscopic study

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ABSTRACT

The objective of this study is to address quantitative microscopic approach for automated screening of erythrocytes in anaemic cases using scanning electron microscopic (SEM) images of unstained blood cells. Erythrocytes were separated from blood samples and processed for SEM imaging. Thereafter, erythrocytes were segmented using marker controlled watershed transformation technique. Total 47 structural and textural features of erythrocytes were extracted using various mathematical measures for six types of anaemic cases as compared to the control group. These features were statistically evaluated at 1% level of significance and subsequently ranked using Fisher's *F*-statistic describing the group discriminating potentiality. Amongst all extracted features, twenty nine features were found to be statistically significant ($p < 0.001$). Finally, Bayesian classifier was applied to classify six types of anaemia based on top seventeen ranked features those of which are of course statistically significant. The present study yielded a predictive accuracy of 88.99%.

1. Introduction

Decrease in the erythrocyte count or decrease in the blood haemoglobin level followed by insufficiency in the oxygen-carrying capacity is generally known as anaemia. In the past few decades, the Eastern Mediterranean Region and Asian Continent showed a considerable growth in anaemia. According to statistics shown by the National Family Health Survey (NFHS-3) in 2005–2006 and WHO (2008), India also showed a significant growth in anaemia in the past few years. The report stated that 70–80% of children, 50% of pregnant women and 24% of men were affected by anaemia in India. Prevalence of anaemia in India is high because of low dietary intake and poor availability of iron and chronic blood loss due to hook worm infestation and malaria.

Anaemia is classified using two approaches (Dan and Lango, 2011): (a) kinetic approach which is based on evaluating production, destruction and loss of erythrocytes and; (b) morphological approach which is based on shape and size of erythrocytes. Our study is based on morphological approach i.e. based on shape and size change of erythrocytes. Size of erythrocytes is proportional to the mean corpuscular volume (MCV) (Dacie and Lewis, 1984). Anaemia is classified into three groups depending on the size of erythrocytes (Fig. 1) viz., (a) microcytic anaemia in which the erythrocytes are smaller than their normal size; (b) macrocytic anaemia in which the erythrocytes are larger than their normal size and (c) normocytic anaemia in which the erythrocytes are of normal size. Sometimes anaemic erythrocytes were paler than usual (Dan and Lango, 2011). In such situation, erythrocyte is known as *hypochromic* if central pallor region of anaemic erythrocyte was wider than normal and known as *normochromic* if the central pallor region is normal. Fig. 1 shows the classification of erythrocytes based on morphological approach.

The evaluation using compound microscopic imaging along with biochemical tests are performed for diagnosis of anaemia. Such procedure is stain variant, time consuming and subjective. In fact, compound microscopic images are also insufficient for proper identification of different groups (macrocytic, macrocytic & normocytic) and sub-group (hypochromic & normochromic) of anaemia (Fig. 1). Usually haematologist can detect major groups of anaemia but it is very difficult to interpret whether it is hypochromic (paler than usual) or normochromic from compound microscope images. Depending upon the central pallor size and shape, experts classify whether the erythrocytes are hypochromic or normochromic, but it is difficult to observe central pallor in compound microscope images. In view of this, there is indeed a requirement to consider a stain invariant imaging technique which will not at all affect the morphology and texture of erythrocytes for providing better information in automated recognition of anaemia. Scanning electron microscopy (SEM) is one of the most advanced imaging techniques, which uses a high energy beam to scan the

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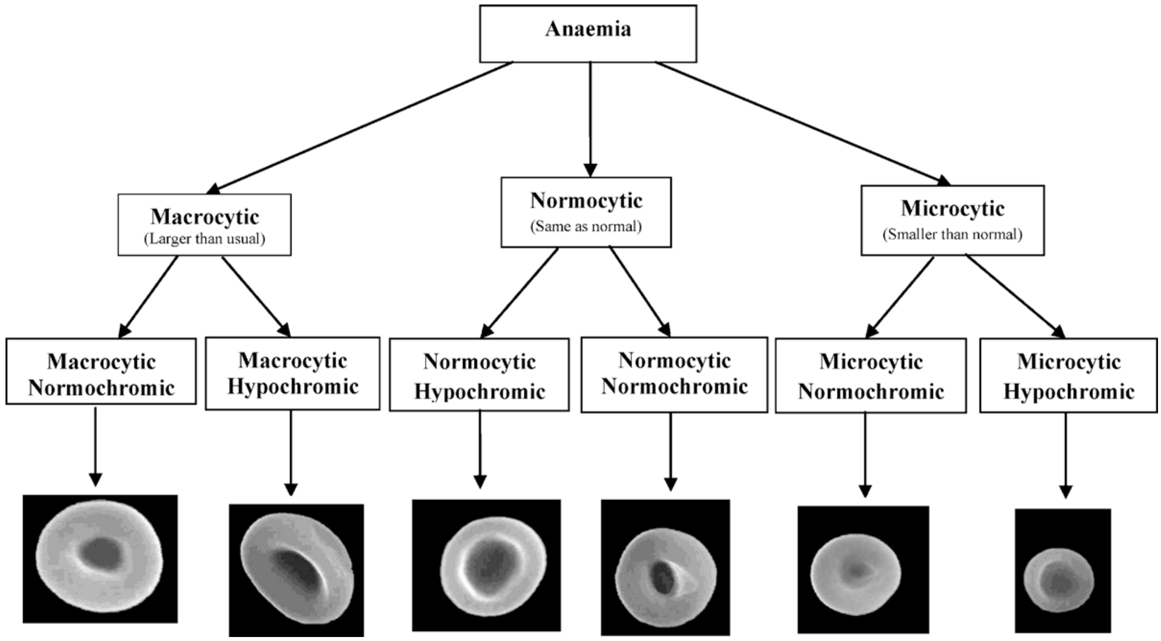

Fig. 1. Morphological classification of anaemic erythrocyte.

object. Textural or morphological changes in erythrocytes can be observed from SEM images. The study aims at developing a computer assisted method for anaemia classification based on textural and morphological changes observed under SEM images of unstained peripheral blood.

Our approach is based on morphology and texture of the erythrocytes i.e. shapes, size, textural and surface texture variation of abnormal erythrocytes. Here, normal and anaemic blood samples were chemically processed and fixed over haemocytometer coverslip pieces. Once SEM imaging was over, the normal and the anaemic erythrocytes were segmented using marker controlled watershed algorithm from SEM images. After considering pathologists opinion, we have selected only anaemic cells out of all images for feature extraction and classification step. Thereafter, geometrical features like area, perimeter, eccentricity and compactness etc. and gray level co-occurrence matrix (GLCM) based textural features were extracted from those correctly segmented images (cells) to identify anaemia through pseudo image construction. All the extracted features intending to characterize seven classes of erythrocytes (six classes of anaemia and normal) were statistically evaluated using probability density function, Box–Whisker's plot and One-way ANOVA. In addition, the significant features were ranked using Fisher's *F*-statistic for systematic classification. Finally, Bayesian approach as one of the statistical learning techniques was trained and tested for predicting particular type of anaemia. Fig. 2 shows the overall flow chart of our study design and methodology.

2. Previous work

In the literatures, most of the works related to automated erythrocytes' classification is based on compound microscopic imaging and image analysis. Bacus et al. (1976) proposed image processing for automated erythrocyte classification using roundness, specularity, eccentricity and central gray level distribution. Multivariate Gaussian distribution along with an interactive graph display was used to separate six distinct condensed subgroups of red cells. Bacus and Weens (1977) showed an automated method of differential red blood cell classification with application to the diagnosis of anaemia. They utilized internal central pallor configurations along with some shape features (area, circularity, elongation, spicularity etc.) for red blood cell classification. They used features with fully automated decision logic to provide a differential analysis of red blood cell. Computer-aided detection technique was used by Masala (2005) to distinguish between carriers of thalassemia and healthy subjects. Principal component analysis (PCA) was used to reduce the number of features by selecting higher eigen-values and corresponding eigen-vectors. Feed-forward neural networks, support vector machine (SVM) and *k*-nearest neighbours (Masala, 2005) were used for a three-class pattern classification problem with a comparative analysis. Hirimutugoda and Wijayarathna (2010) used images of thalassemia and malaria parasites from light microscopic blood smear for rapid and accurate automated diagnosis of RBC disorders and also described a method to detect malarial parasites and thalassemia in blood sample. Back propagation artificial neural network with image analysis technique were used to evaluate the accuracy of the classification in the recognition of medical image patterns associated with morphological features of erythrocytes in the blood. Contour tracing approach was used by Vromen and McCane (2009) for segmenting SEM image of normal RBC. They used a second order polynomial model with a simple Bayesian approach, and a post process ellipse fitting procedure to ensure smooth boundaries and cull noise contour, respectively. It is noticed from the literatures that there were no attempt except Vromen and McCane (2009) to develop automated anaemia classification using SEM images of erythrocytes.

3. Materials and methods

3.1. Sample collection and slide preparation

The study subjects were considered in this study for normal and anaemic groups followed by examining the normal (for female above 12 g/dl, for male above 13 g/dl) and abnormal (for female below 11 g/dl, for male below 12 g/dl) (WHO, Geneva, 2008) haemoglobin levels at Midnapur Medical College & Hospital and Medipath Laboratory, West Bengal, India. Thereafter, peripheral blood samples were collected in EDTA vial to avoid coagulation. To reduce the deformation of erythrocytes caused by EDTA, aliquot of