Oral Exfoliative Cytology In Beta Thalassaemia Patients Undergoing Repeated Blood Transfusions

S Nandaprasad, P Sharada, M Vidya, B Karkera, M Hemanth, N Prakash

Citation

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Abstract

 -Thalassaemia [thalassaemia major] causes severe transfusion dependent anemia. In the heterozygous state, the -Thalassaemia trait [thalassaemia minor] causes mild to moderate microcytic anemia. Our study comprised of hundred -Thalassaemia major patients undergoing a minimum of 15 transfusions and hundred normal individuals. Both the control and study group excluded patients with iron deficiency anemia, megaloblastic anemia, malignancy and chronic liver damage. Exfoliated cells from the buccal mucosa of 65 out of the 100 -Thalassaemia major patients revealed positivity for Perl's Prussian blue reaction. Applying the chi-square test to compute the p-value, from the computations it is evident that p<.005. We also used the chi-square test to determine whether the Perl's Prussian blue reaction is gender based. Out of the 65 positive patients of the study group, 40 were females and 25 were males. From the computations we found that p>0.005. Hence, we concluded that Perl's Prussian blue reaction test is not gender based.

INTRODUCTION

Iron is an essential nutrient required by every human cell. Approximately, 4.5g of iron can be found in the average adult, most of which is contained in the hemoglobin molecule and other heam-containing protein. [1] Under physiologic conditions, the concentration of iron in the human body is carefully regulated and normally maintained at approximately 40 mg iron/kg body weight in women and approximately 50 mg Fe/kg body weight in men, distributed between functional, transport and storage components. [2]

The regulation of intestinal absorption of iron plays a crucial role to satishfy the needs of erythropoiesis.it is critical because humans have no physiologic pathway for excretion,except recurrent blood loss through menstruation and desquamation of enterocytes(cycle of enterocytes is about 35 hours). Duodenal crypt cells sense the iron requirement of the body and the needs of erythropoiesis are programmed by that information as they mature into absorptive enterocytes. Enterocytes lining the absorptive villi close to the gastro-duodenal junction are responsible for all iron absorption. [3] the iron balance at the cellular level is obtained by opposite regulation of the synthesis of ferritin and transferring receptors(TFR). [4]

In 1925, Thomas Cooley and Pearl Lee described a form of severe anemia, occurring in children of Italian origin, associated with splenomegaly and characteristic bone changes. As all earlier cases were reported in children of the Mediterranean origin, the disease was later termed thalassaemia, from the Greek word for sea, thalassa. Over the next 20 years, it became apparent that Cooley and Lee had described the homozygous or compound heterozygous state for a recessive Mendelian disorder not confined to the Mediterranean, but occurring widely throughout tropical countries. In the past 20 years, the two important forms of this disorder, and -thalassaemia, resulting from the defective synthesis of the and -globin chains of hemoglobin, respectively, have become recognized as the most common monogenic diseases in humans.

This article focuses on -thalassaemia major, which by far is the most important of all the thalassaemias.

In -Thalassaemia major [i.e., homozygous -Thalassaemia], the production of -globin chains is severely impaired, because both -globin genes are mutated. The severe imbalance of globin chain synthesis result in ineffective erythropoiesis and severe microcytic hypochromic anemia. The excess of unpaired globin chains aggregate to form precipitates that damage red cell membranes, resulting in intravascular hemolysis. Premature destruction of erythroid precursors result in intramedullary death and ineffective erythropoiesis. The profound anemia is typically associated

with erythroid hyperplasia and extramedullary hematopoiesis. [5]

Chronic blood transfusion is associated with many untoward complications like blood borne infections, isoimmunisation, febrile reactions and iron overload.

Normal persons require 1 to 2 mg/l of elemental iron, an amount readily delivered by most diets. A unit of packed RBCs contain 250 to 300 mg iron [1mg/ml], so that a single transfusion of two units of packed RBCs is about equal to a 1 to 2 year intake of iron. There are no mechanisms for increasing the excretion of iron beyond normal daily losses. Iron thus rapidly accumulates in chronically transfused patients.

Common clinical complaints in iron overload include lethargy, weight loss, change in skin color, loss of libido, abdominal pain and joint pain.

Iron accumulation in -Thalassaemia major patients depends upon the number of blood transfusions given. One unit of blood contains 250 ml of RBCs. Since 1ml of RBC contains 1mg of iron, 4 units of blood will contain around 1gm of iron. Signs of clinical toxicity become apparent, when body iron reaches 400 to 1000mg/kg body weight. [6] Signs of iron overload can be usually seen after 10-12 transfusions. [7]

Since serum ferritin level directly reflects the body iron status in the normal human subject, it is used as a routine test in the diagnosis of iron overload and monitoring the response to treatment. However, serum iron concentration is increased in some diseases even when the body iron stores are within normal limits, as in acute and chronic liver damage, malignancies, infections and megaloblastic anemia. Liver and bone marrow biopsy are as such invasive procedures and thus cannot be used for frequent assessment of iron overload or for screening purposes [6]

Exfoliative cytology is the microscopic examination of cells shed or desquamated from the epithelial surface, usually the mucous membrane. It also includes the study of those cells, which have been collected from body fluids such as sputum, saliva etc. It is a simple, painless, bloodless and quick procedure. [8]

A clear clinical need is evident for quantitative, noninvasive, safe, accurate, and readily available means of measuring body storage iron to improve the diagnosis and management of patients with iron overload from such disorders as hereditary hemochromatosis, thalassaemia major, sickle cell disease, aplastic anemia, and myelodysplasia, among others.

The objective of this study was to know whether oral exfoliative cytology using Perl's Prussian blue staining, can be a tool in -thalassaemia major patients undergoing repeated blood transfusions.

METHODOLOGY

Study group consisted of 100 -thalassaemia major patients undergoing repeated blood transfusions of at least 15, they were selected through purposive sampling technique from St. John's medical college Hospital, Bangalore. Control group consisted of 100 healthy individuals, the study duration was for 1 ½ years. Both the control and study group excluded persons with iron deficiency anemia, megaloblastic anemia, malignancy and chronic liver damage.

Exfoliated epithelial cells were taken from the buccal mucosa of the oral cavity. For this, first the patients from the study group and the control group were asked to gargle their mouth in distilled water. The buccal mucosa of the patients were scraped with a wet wooden spatula and smeared onto glass a glass micro slide. The smear was fixed immediately in 70% ethanol for one hour and then stained with Perl's Prussian staining kit, which consists of potassium ferrocyanide, which reacts with the ferritin in the cells to form a blue color, 0.5% aqueous neutral red was used as the nuclear stain. Blue granules indicated presence of iron overload. The stained smear was examined under the light microscope through the transmitted light at 10x, 40x and 100x magnifications to study the presence or absence of blue colored granules in the cells. Chi-square test was used for comparing the study group and the control group.

Figure 1

Fig 1: photomicrograph showing positivity for Perl's Prussian stain

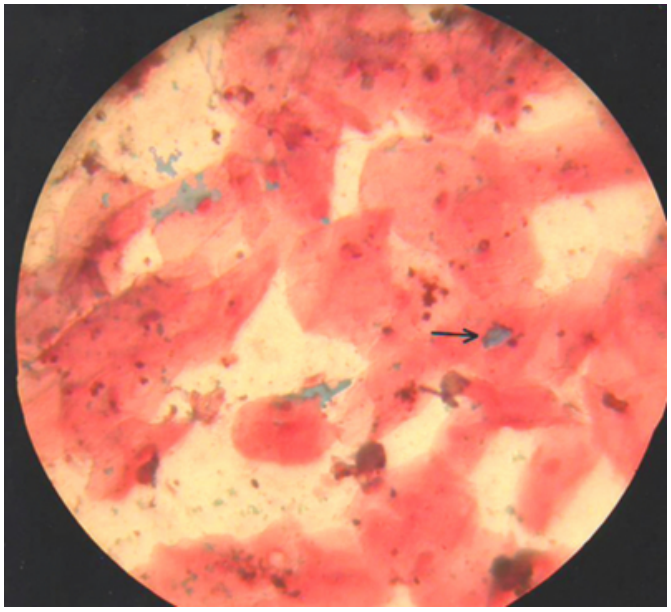


Figure 2

Fig 2: photomicrograph showing positivity for Perl's Prussian stain

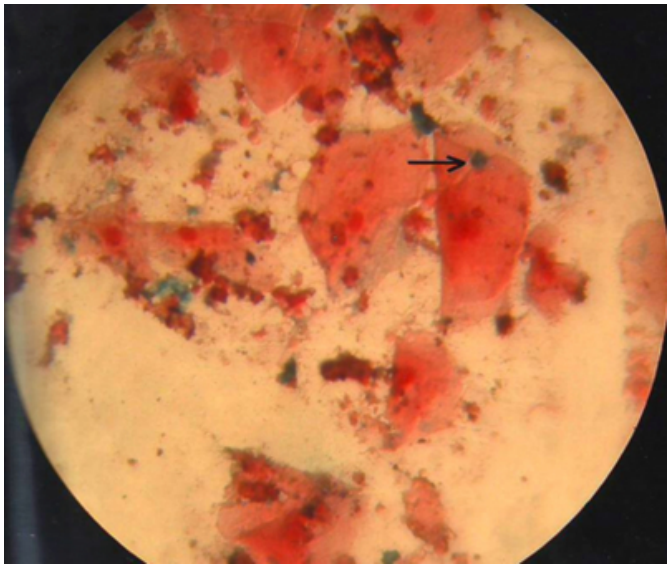
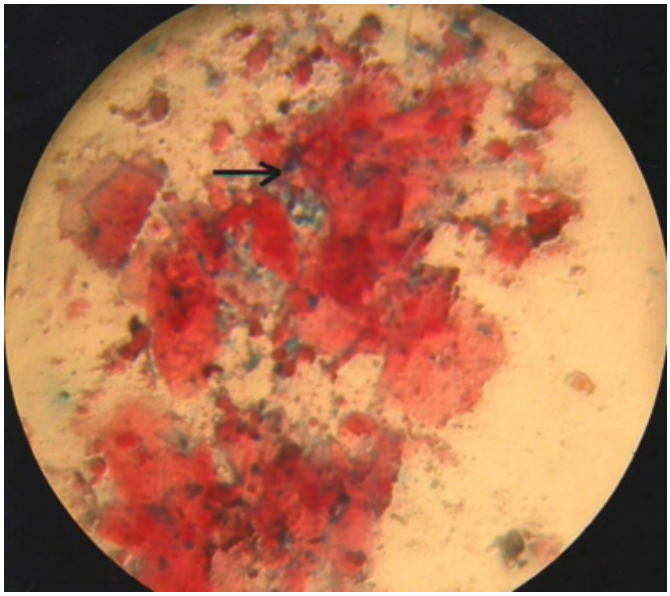


Figure 3

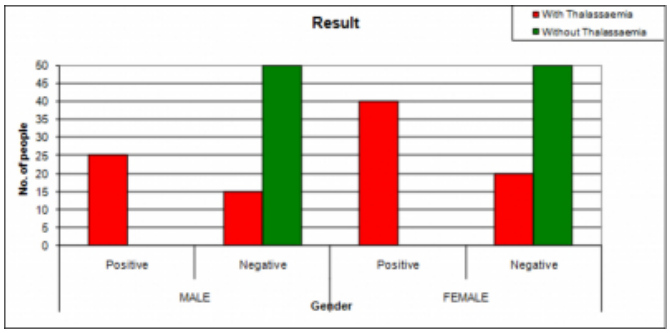
Fig 3: photomicrograph showing positivity for Perl's Prussian stain



RESULTS

The results are shown in the following graph:

Figure 4



DISCUSSION

In our study, exfoliated cells from the buccal mucosa of hundred -Thalassaemia major patients comprising the study group undergoing a minimum of 15 transfusions and a control group containing hundred normal individuals who had no confirmed acute and chronic liver damage, malignancy and megaloblastic anemia were considered and the smears obtained were stained with Perl's Prussian blue stain.

Perl's Prussian blue reaction is considered to be the first classical histochemical reaction, carried out, and is widely applied in the field of hematology. [2] Treatment with a dilute acid is an essential preliminary in the performance of the Prussian blue reaction. By this means, the ferric iron is

liberated from unreactive loose combinations with proteins such as hemosiderin. We have applied this technique to exfoliated buccal mucosal cells, considering the fact that the exfoliated cells possibly represent changes in the underlying parent tissue.

Patients with acute and chronic liver damage, malignancy, infections and megaloblastic anemia were excluded from the present study as these patients show an increased serum iron concentration even when the body iron stores are within normal limits as followed in a similar study by Gururaj, Sivapathasudaram (2004) [2]

Exfoliated cells from the buccal mucosa of 65 of the 100 thalassaemia patients in our study group revealed positivity for Perl's Prussian blue reaction conducted. Statistically the 'p' value is < 0.005, and the test is considered significant. A similar study by Gururaj, Sivapathsundaram [2004] [6] on ten patient's revealed positivity for Perl's Prussian blue reaction. But in both the studies, none of the patients in the control group showed positivity for the Perl's Prussian blue reaction. In our study out of 65 positive patients, 40 were women and 25 were men. Our statistical analysis concluded that 'p' value was >0.005, which was insignificant.

CONCLUSION

The objective of this study was to establish oral exfoliative cytology as an ideal screening and diagnostic tool in -thalassaemia major patients undergoing repeated blood transfusions. Owing to the smaller sample size and lack of correlative Perl's staining in tissue biopsies in our cases, the diagnostic reliability of Perl's Prussian blue reaction in oral exfoliated cells to demonstrate iron overload is still debatable.

By considering the simplicity and acceptability of exfoliative cytology methods, further studies on correlating Perl's Prussian blue reaction to serum ferritin levels and MRI images can establish this non-invasive procedure as an ideal screening and diagnostic tool in all patients undergoing repeated blood transfusions and also to assess future complications associated with iron overload and thereby institute appropriate treatment.

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Author Information

S. Nandaprasad, MDS
Assistant Professor, Dept.of Oral Pathology, Yenepoya Dental College, Mangalore

P. Sharada, MDS
Professor & Head, Dept.of Oral Pathology, Maruthi Dental College,Bangalore

M. Vidya, MDS
Professor & Head, Dept.of Oral Pathology, Yenepoya Dental College, Mangalore

Bhavana.V. Karkera, MDS
Assistant Professor, Dept.of Oral Pathology, Yenepoya Dental College, Mangalore

M. Hemanth, MDS
Assistant Professor, Dept.of Oral Pathology, Yenepoya Dental College.

Nilima Prakash, MDS
Assistant Professor, Dept.of Oral Pathology, Maruthi Dental College,Bangalore