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ORIGINAL ARTICLE

Cytological Detection of Iron Overload and Cytomorphometric Changes in Buccal Mucosa of Beta Thalassemia Major Patients

NASIR KARIM[1], GHAZIA FATIMA[3], ZAFAR IQBAL[3], TAHREEM ANWER[4], FAIZ AHMAD FAIZ[5]
[1]*Pathologist, DHQ Hospital, Nankana Sahib*
[2]*Assistant Professor Histopathology, Continental Medical College, Lahore*
[3]*Associate Professor Pathology, Continental Medical College, Lahore*
[4]*Sr. Demonstrator Hematology, Continental Medical College, Lahore*
[5]*Assistant Professor Hematology, Islam Medical College, Sialkot*
Correspondence to Dr. Nasir Karim, Email: drkarim.nasir@gmail.com

ABSTRACT

Aim: In this study, the phenomenon of oral exfoliative cytology is explored and buccal squamous cells are examined for intracytoplasmic, Prussian blue stained, iron containing granules. In addition, these buccal squamous cells are subjected to morphometric measurements of their nuclear and cytoplasmic diameter along with their ratios.
Methods: It was a Descriptive, Cross sectional study including 120patients of Thalassemia Major, ranging from 4 -18 years with repeated blood transfusions and a serum ferritin level of 1000ng/ml at minimum. Scrapings were obtained from buccal mucosa for cytological examination and stained with Prussian Blue Stain.
Results: The study included 45 female and 75 male patients with a mean age 11.04(± 3.81) years. 56out of 120 patients were positive for Prussian blue and thus showed 46.6% positivity. The mean cytoplasmic diameter was 50.90 (± 0.64) microns and mean nuclear diameter was 9.31(±0.53) microns. Mean Nuclear to cytoplasmic (NC) ratio was 0.182(± 0.01) and ranged from 0.165 and 0.211 microns.
Conclusion: The positivity of Prussian blue in our study is clearly less than most of the quoted figures while alterations in morphometric features of buccal squamous cells are quite consistent with reported data. In our study, we observed iron containing granules not only in the cytoplasm but also on surface of cells like a clue cell dispersed outside the cell, individually or in discrete clusters and as faint bluish hue in background.
Keywords: Iron overload, Cytomorphometry, buccal smear, Prussian blue, Beta Thalassemia

INTRODUCTION

About 300,000 children are born each year with inherited disorders of hemoglobin across the world and most of them reside in developing countries with poor socio economic indicators[1]. Among these hemoglobinopathies, Sickle cell anemia is the most prevalent, comprising 2/3rd of the cases, while Thalassemia is the 2nd most common disorder[2].These Hemoglobin disorders account for about 3.4% of deaths in children less than 5 years of age[3]. The incidence of Thalassemia is about 4.4 for every 10,000 live births with no gender predisposition and only individuals with major form of disease, seek regular medical attention with an average life expectancy of 17 years[4].

It is a diverse group of disorders with variable, worldwide distribution and currently restricted to three types of countries. First are the once Endemic Mediterranean countries where80%-100% prevention has been claimed to be achieved now with some quoting an average frequency of 7.6% in the modern Cretan population[5]. It is worth mentioning that Thalassemia, in addition to other hemoglobinopathies confers a protective role against malaria with variable degree of protection and presents as a model for investigation and research in malaria pathogenesis[6].

METHODS

This descriptive, cross sectional study was conducted at King Edward Medical University, Lahore during a period of six months. All the patients came for routine blood transfusions at Thalassemia Center, Sir Ganga Ram Hospital and Children Hospital, Lahore. Written and verbal permission was taken in advance from both parent institute and Thalassemia Center as well. Sample size was 120 patients. Sampling technique used was non probability, purposive sampling.

Inclusion Criteria

- Confirmed cases of β–thalassemia major.
- Patients in age group of 4 -18 years.
- Repeated blood transfusions and a serum ferritin level of minimum 1000ng/ml

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Exclusion Criteria: Patients with any systemic disease or those who are on therapeutic medication

Variables included in the study: Nuclear diameter or size (ND), Cytoplasmic diameter (CD), Nuclear to cytoplasmic (N/C) ratio.

It comprised a total of 120 confirmed cases of beta thalassemia major ranging in the age group of 4- 18 years who are receiving regular blood transfusions. Their clinical record, Hb electrophoresis reports and a cutoff of serum ferritin level i.e. 1000 ng/ml was made sure to include only beta thalassemia major patients. Scrapings were obtained for cytological examination. At least six smears were prepared from each case, two of them were stained with Prussian blue stain (Perl's Reaction) to demonstrate the iron in the cytoplasm of buccal squamous cells and the remaining were stained with PAP Stain for cyto-morphometric analysis (to measure nuclear and cytoplasmic sizes along with their ratio, using a simple ocular micrometer).

Findings:

- Iron (hemosiderin): blue
- Nuclei: red
- Background: pink

All the observed values were subjected to statistical analysis using SPSS version 22.

RESULTS

The mean age of 120 thalassemia patients included in the study, while fulfilling the inclusion and exclusion criteria, was 11.04 years with ± 3.81 SD. The minimum age of the patients included in the study was 4 years and the maximum age was 19 years (11.04±3.81 SD). As a group, 7 to 9 years old patients were most frequent, comprising 37 cases of the total 120 cases, with a relative frequency of 30.83%.

The mean cytoplasmic diameter in all 120 thalassemia patients after examining 20 cells from every single case was 50.90 (± 0.64)microns. Regarding cytoplasmic diameters, the largest cell measured 52.55 microns and the smallest one measured 49.55 micron (Mean 50.90±0.64, Table 2). Cells ranging from 50.55-51.05 micron comprised the most frequent group with 31% relative frequency. The mean nuclear diameterof 9.31(±0.53) microns was calculated, with largest nucleus measuring 10.56 and the smallest one calculated to be 8.35micron. 8.95 micron presented the mode

value while nuclear diameters ranging from 8.85-9.35 micron represented the most frequent group constituting 49 patients out of a total of 120 patients having a relative frequency of 41%.

The mean nuclear to cytoplasmic (NC) ratio was 0.182(±0.01SD). The ratios varied as 0.046 between 0.165microns and 0.211 microns. Buccal smears of 56 out of 120 cases were positive for Prussian blue or iron stain which conferred 46.6 % positivity. Regarding gender distribution the study included 45 female and 75 male patients, which constituted 38 and 62 percent of total sample strength respectively (Table 6).Out of the 56 Prussian blue positive smears, 20 cases belonged to female patients while remaining 36 were from male patients calculated as 44% and 48% positivity in each gender respectively.

Table 1: Age Distribution

Age in 3 year intervals	Frequency	%age
4-6	13	10.83%
7-9	37	30.83%
10-12	24	20.00%
13-15	29	24.17%
16-19	17	14.17%
Total	120	100.00

Table2: Descriptive data of qualitative variables

	Age(Yrs)	CD microns	ND microns	N/C Ratio
Mean	11.04	50.90	9.31	0.182
Median	11	50.9	9.1	0.179
Mode	8	50.6	8.95	0.173
SD	3.81	0.64	0.53	0.01
Range	15	3	2.21	0.046
Minimum	4	49.55	8.35	0.165
Maximum	19	52.55	10.56	0.211

Table 3: Mean Cytoplasmic diameters (CD)

CD intervals	n	%age
49.55-50.05	12	10%
50.05-50.55	21	18%
50.55-51.05	37	31%
51.05-51.55	28	23%
51.55-52.05	19	16%
52.05-52.55	3	3%
Total	120	100%

Table 4.Mean nuclear diameters (ND)

ND Intervals	n=	%age
8.35-8.85	20	17%
8.85-9.35	49	41%
9.35-9.85	24	20%
9.85-10.35	21	18%
10.35-10.85	6	5%
Total	120	100

Table 5: Mean Nuclear to cytoplasmic ratio

N/C ratio at 0.01 interval	n	%age
0.165-0.175	39	33%
0.175-0.185	35	29%
0.185-0.195	27	23%
0.195-0.205	17	14%
0.205-0.215	2	2%
Total	120	100%

Table 6: Male and female subjects.

Gender	n	%age
Females	45	38%
Males	75	62%
Total	120	100%

Table 7: Prussian blue positivity

Prussian blue reaction	n	%age
Negative	64	53.33%
Positive	56	46.66%
Total	45	100%

Table 8: Gender wise prussian blue positivity

Prussion blue reaction	n	%age	n	%age
Negative	25	56%	39	52%
Positive	20	44%	36	48%
Total	45	100%	75	100%

DISCUSSION

The mean age of 120 thalassemia major patients included in our study, was 11.04 years with ± 3.81 SD. The age ranged from 4 to 19 years. As far as gender is concerned, the study included 45 female and 75 male patients, which constituted 38 and 63 percent of the total sample strength respectively. Buccal smears of 56 out of 120 patients revealed positivity for Prussian blue or iron stain conferring 46.6 % positivity overall with 20 cases belonging to female patients while the remaining 36 were from male patients, leading to 44% and 48% positivity in each gender respectively. The mean cytoplasmic diameter was 50.9(±0.64 SD) micron, ranging from 49.6 to 52.6 micron while themean nuclear diameter was calculated to be 9.3(±0.53 SD) micron. The ratio of these two parameters, i.e. mean Nuclear to cytoplasmic (NC) ratio was 0.182(± 0.01 SD) and ranged from 0.165 to 0.211 microns.

Our study revealed 46.6 % Prussian blue or iron stain positivity, which is comparatively lower than most of the studies mentioned in literature. There are reports of Prussian blue positivity ranging from around 60% to complete 100% with almost all showing a significant p value, when compared to their perspective control groups. Lowest positivity was calculated by Gupta et al[9] who found 61.6% positivity. Bhat et al[7] came up with 71.7% positivity for 43 cases out of 60 thalassemia patients while another study, 82.9% of cases showed positivity for Perl's Prussian blue. Chittamsettyetal[8] observed 72.5% positivity while studying buccal smears from patients of another hemoglobinopathy i.e. Sickle Cell anemia. It is worth noting that in all of the above mentioned studies, none of the patients from control groups showed positivity for the Prussian blue. Although it was assumed that oral epithelial cells reflect the changes in the liver tissue with an iron overload and most should show positivity for Prussian blue. Instead there is considerable variation in reported percentage positivity of Prussian blue, which is itself confounding and needs an explanation. In addition, the results of present study revealed further decrease in positive staining.

The mean cytoplasmic diameter in our study was 50.9 (± 0.64) micron while mean nuclear diameter of same cell was 9.3 (±0.53) micron, with resulting Mean Nuclear to cytoplasmic (NC) ratio of 0.182 (±0.01).In contrast to all contemporary studies we did not include a control group in our study, and we compared our results with essentially normal subjects. The mean Cytoplasmic diameter of an average normal buccal squamous cell, given in literature is 51.78 (± 0.11) which is slightly more than our result i.e. 50.90 (±0.64) micron, while the mean nuclear diameter is 8.36 (± 0.49) microns which is 0.95 micron less than our value of 9.31 micron (±0.53)[10].

The difference in results might appear quite subtle on overlook but if we compare the nuclear to cytoplasmic ratio of normal cells as 0.161 with 0.182 in our study, there is an increase in nuclear to cytoplasmic ratio. It can be deduced from these figures that with more or less normal cytoplasmic diameter, there is increase in nuclear size and subsequently, nuclear to cytoplasmic ratio. This slight but consistent increase confers that beta thalassemia patients have comparatively increased nuclear to cytoplasmic ratio, which may vary up to 0.211. It can be fairly assumed that patients with increased iron overload have higher nuclear to cytoplasmic ratio of their buccal squamous cells but concluding that these cyto-morphometric measurements can be used as a screening or monitoring method in thalassemia patients for iron overload is quite an oversimplification.

From the above mentioned discussion it is evident that cytomorphometry has a definite role in diagnosis and

management of generalized metabolic conditions like DM and Thalassemia, at least statistically and in research papers.

CONCLUSION

The positivity of Prussian blue in our study is clearly less than most of the quoted figures while alterations in morphometric features of buccal squamous cells are quite consistent with reported data. In our study, we observed iron containing granules not only in the cytoplasm but also on surface of cells. The method of cyto-morphometry proved to be efficient, as far as statistics are concerned, and our results are in accordance with previous studies but to accept its role as a diagnostic tool to detect iron overload in beta Thalassemia major patients is difficult.

Conflict of interest: Nil

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