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SICKLE CELL TRAIT FREQUENCIES IN BLOOD DONORS AND ITS EFFECT AMONG RECEIPIENTS IN BUNGOMA COUNTY, KENYA

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ABSTRACT

Sickle cell trait blood can cause complications in transfused patients.

Objective: This study aimed at evaluating the prevalence of sickle cell trait among the blood donors and its impact in transfused patients in Bungoma County.

Design: A cross sectional study targeting healthy blood donors and patients transfused with sickle cell trait blood in Bungoma County from January 2019 to January 2020.

Participants: A total of 350 blood donors and 10 patients transfused with sickle cell trait blood were enrolled.

Results: The sickle cell trait prevalence amongst the blood donor population in Bungoma County was deduced to be 14.28 %. White, red blood cell counts, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration parameters were not affected in patients transfused with sickle cell trait blood ($p = 0.466, 0.980, 0.787, 0.886, 0.971, 0.476$ and 0.524). Also, liver function tests in the same patients was not affected ($p = 0.2193, 0.4678, 0.3052$ and 0.7263) respectively for Alanine, Aspartate, Direct and Total Bilirubin levels respectively.

Conclusion: There were no clinical abnormalities in patients transfused with sickle cell trait, however it was observed that acquired haemoglobin AS (Sickle cell trait) was detected among the transfused population.

INTRODUCTION

Blood transfusion is a therapy that saves lives; however, every transfusion carries a possibility of an adverse event in the recipients¹. Among such hazards includes induced haemoglobinopathies which are associated with wrong diagnosis, expensive testing and counselling of patients.² Other hazards associated with blood transfusion are viral infections, mild to severe transfusion reactions and alloimmunisation.³

Sickle cell trait is caused by inheritance of haemoglobin S in heterozygous form and its phenotypic expression is not symptomatic hence people carrying this gene end up qualifying to donate blood.⁴ Sickle cell trait blood is harmful to patients particularly those suffering from sickle cell disease.⁵

The prevalence of sickle cell trait varies in various parts of the world. For instance, in the United States of America, 140,000 units of blood collected every year are from sickle cell trait individuals.⁶ A high prevalence of sickle cell trait of 27.1% has been documented in Nigeria.⁷ In Kenya, Haemoglobin S carrier frequency among adolescents youths has been reported to be 15% most this group are potential blood donors.⁸

Some studies have shown normal or increased red cell indices in patients transfused with abnormal haemoglobin variants. For instance, in Italy, a diabetic female patient transfused with haemoglobin J variant had her red blood cell indices increase from 2.50 to 3.35×10^{12} per microliter, 6.8 to 9.6 grams per deciliter and 21.8% to 29.0% for red cell count, haemoglobin and haematocrit levels respectively.⁹

Also, in the USA, a patient who had also been transfused with G- Philadelphia and Haemoglobin A2 variants had also an increase of red blood cell count from 2.2 to 3.39×10^{12}

per microliter, haemoglobin level increased from 7.5 grams per deciliter to 9.8 grams per deciliter while haematocrit levels rose from 22.1% to 28.6% .¹⁰ This clearly demonstrates that blood collected from sickle cell trait individuals do have quality parameters similar to those of sickle cell trait negative blood donors.¹¹

On the other hand, lack of adverse reactions in beta thalassemia patients transfused with haemoglobin AS variant blood in India has also been documented.¹² Despite the rare hazards associated with haemoglobin variants in transfused patients, misdiagnosis in post transfused patients is costly, time wasting and leads to wrong notification and patient management.²

Therefore, this study aimed at determining the frequency of sickle cell trait among blood donor population and its impact in transfused patients in Bungoma County. The findings of this study will play a great role in patient management and health planning especially in controlling the burden of sickle cell disease in future generations through counseling of the affected blood donors.

MATERIALS AND METHODS

Study area: The study was carried out between January 2019 to January 2020 in Bungoma County (coordinates 0.4213°N to 1.1477°N along the latitude and 34.3627°E to 35.0677°E along the longitude) in Western Kenya. This was a good study area because sickle cell patients who are vulnerable to sickle cell trait transfusions account for 12% of transfusions carried out in Bungoma County.¹³

Study Population: All individuals who met the standard criteria of blood donation which included haemoglobin level of over 12.5 grams per decilitre, over 50 kilograms of weight, not breast feeding and expectant for female blood

donors, not suffering from chronic illness were enrolled in the study. Individuals with low haemoglobin, weight, suffering from chronic illnesses and unhealthy lifestyle were excluded from the study. All patients transfused with sickle cell trait blood in Hospitals with appropriate blood bank equipment had their pre and post haematological and biochemical blood profile evaluated.

Study design: The study was cross sectional and involved the screening of volunteer blood donors for sickle cell trait status and pre - and post - transfusion testing for full blood count and liver function test of patients transfused with sickle cell trait blood.

Laboratory procedures

Sample collection and analysis: Blood samples from volunteer blood donors for Sickle cell trait screening were collected in EDTA vacutainer tubes. Pre- and post- transfusion whole blood samples for full blood count and High performance liquid chromatography (HPLC) tests were collected from patients transfused with sickle cell trait positive blood in EDTA vacutainer tubes while for liver function tests blood samples were collected in plain tubes respectively. Serum liver function test was immediately separated by centrifugation from whole blood samples.

i. Sickle cell trait screening – Sodium Metabisulphite method

Sickle cell trait was screened using Sodium metabisulphite method.⁴ Briefly, two drops of freshly prepared 2% sodium metabisulphite was added to another two drops of thoroughly mixed EDTA anticoagulated blood and mixed on a clean microscope slide, which was then covered with a clean coverslip, sealed with paraffin wax and incubated at room temperature thirty minutes. The results were interpreted as positive by the presence of typical sickle-shaped red blood cells and as

negative by the absence of typical sickle-shaped red blood.⁴

ii. Pre – and post – transfusion full blood counts in patients transfused with sickle cell trait blood.

Full blood count was tested using Medonic M32M haematology analyser. Briefly, the procedure involved pressing numerical on the screen to enter sample identification details; placing well-mixed samples on the autoloader; pressing the start icon on the screen to start sample aspiration and analysis; the results were displayed on the screen and automatically printed immediately the instrument completed the analysis.

iii. High performance liquid chromatography

High performance liquid chromatography technique was used following previously used protocol to determine haemoglobin variants. Whole blood (5 microliters) were mixed with 1.0 millilitres of diluent and then injected into a special cartridge for processing.¹⁴ The generated chromatograms were interpreted according to the manufacturers' recommendations.¹⁴

iv. Liver function tests

Liver function tests (Total bilirubin, direct bilirubin, alanine transferase and aspartate transferase) were analysed by use of Human star 300 Biochemistry analyser (HUMAN Gesellschaft für Biochemica and Diagnostica mbH Max-Planck-Ring 2165205 Wiesbaden, Germany) as previously used. Briefly, 200 microliters of serum obtained from whole blood collected in a plain vacuitainers were run on the HUMAN 300 Biochemistry analyzer alongside controls.

Quality Assurance of the data: To ensure that quality of data collected pre-donation requirements accordance with the blood transfusion donor guidelines were applied during the study.

Data Management and Analysis: Data collection form was coded, and entries done in Microsoft

excel sheet and analysed using statistical package for social sciences (SPSS V.23) (IBM Corporation, Chicago, Illinois, United States).

Ethical Considerations: The study was approved by the Ethical committee of Baraton University (#REC: UEAB/02/01/2019) and authority to carry out the research was issued by the National Commission for Science, Technology and Innovation (#NACOSTI/P/19/14169/27798). Approval was also sought from the department of health,

Bungoma County (Ref.CG/BGM/CDH/RESRC/VOL.111).

RESULTS

Demographic profile of blood donors

A total of 350 participants were enrolled in the study. Males comprised of 223 (63.7%) while females 127 (36.3%) of the study population. The youngest and the oldest blood donors in the study were 16 and 54 years old respectively (Table 3.1).

Table 3.1

Distribution of blood donors by age category in Bungoma County, Kenya.

AGE GROUP	FREQUENCY	PERCENTAGE
16-20	185	52.86
21-30	84	24
31-40	45	12.86
41-50	22	6.29
51-60	14	4

The distribution of haemoglobin variants against the categories of blood donors in Bungoma County
The distribution of Hb AA variant was more prominent among the first time, followed by repeat and lastly regular blood donors with incidences of 67.71 %, 17.43% and 0.29%

respectively. The frequency of sickle cell trait (Hb AS) in the same blood donor categories was 3.14%, 11.14% and 0 % respectively. Haemoglobin AC and SS were not present among blood donors (Table 3.2).

Table 3.2

The distribution of haemoglobin variants among first time, repeat and regular blood donors in Bungoma County

Blood donor type	Total tested	Hb AA	Hb AS	Hb AC	Hb SS
First time	249	237(67.71%)	11(3.14%)	0(0%)	0(0%)
Repeat	100	61(17.43%)	39(11.14%)	0(0%)	0(0%)
Regular	1	1(0.29%)	0(0%)	0(0%)	0(0%)

Haemoglobin variants distribution among blood donors per gender in Bungoma County

The frequency of Hb AA in male and female blood donors was 55.14 % and 30.57 %

respectively. The distribution pattern for Haemoglobin AS that is responsible for sickle cell trait was 8.57 % and 5.71 % for male and female blood donors respectively (Table 3.3).

Table 3.3*Haemoglobin variants distribution among blood donors per gender in Bungoma County*

Gender	Hb AA	Hb AS	Hb AC	Hb SC
Males	193(55.14%)	30(8.57%)	0(0%)	0(0%)
Females	107(30.57%)	20(5.71%)	0(0%)	0(0%)
Total	300(85.71%)	50(14.28%)	0(0%)	0(0%)

Full blood counts in patients transfused with sickle cell trait blood

During the study period, a total of ten (10) patients were found to have been transfused with sickle cell trait blood during the study period. Apart from two patients 7 and 9 whose haemoglobin level dropped after transfusion, the remaining eight had an increase of haemoglobin between 0.3 to 1.5gms/dl as shown in table 3.4 below. Red cell counts also increased in the eight (8) patients apart from the two patients' number 7 and 9 whose red cell counts reduced by 1.92 and 0.46

respectively (Table 3.4). On white blood cell counts, two patients' number 1 and 7 had an increase while seven had a decrease in total white blood cell counts.

Independent samples t-test was used to compare means for (WBC, RBC, HGB, HCT, MCV, MCH and MCHC) pre and post transfusion with sickle cell trait blood. As indicated in Table 3.5 on average, there was variation in mean parameters pre and post blood transfusion. However, the variation was not statistically significant (all $p > 0.05$) as shown in Table 3.5.

Table 3.4*Pre- and post- transfusion haematological profile in patients transfused with sickle cell trait blood*

Pre transfusion								Post transfusion							
PT	WBC	RBC	HGB	HCT	MCV	MC H	MCH C	WB C	RBC	HGB	HCT	MCV	MCH	MCHC	
1	12.2	4.42	10.2	29.6	67	23.1	34.5	16.4	4.95	11.7	32.8	66.3	23.7	35.5	
2	7.6	4	9.7	30.9	77.2	24.3	31.4	7.5	4.32	10.5	31.9	73.8	24.4	33.1	
3	7.6	3.58	7.7	22.1	61.9	21.5	34.7	7.6	3.91	8.7	25.4	64.8	22.3	34.4	
4	6.69	2.19	4.6	16.2	74	21	28.3	5.23	2.56	5.8	20.2	78.7	22.6	28.7	
5	33.7	2.75	8.2	24.9	90.8	30	23	24.8	2.82	8.5	24.8	87.7	30.1	24.2	
6	5.92	2.31	6.7	25.2	108.9	28.7	26.4	4.67	2.7	7.5	28.8	106.7	27.9	26.1	
7	8.71	6.63	13.8	51.3	75.1	20.2	26.8	11.1	4.71	10.1	35.3	74.8	21.3	28.5	
8	8.82	3.67	7.9	30.2	82.1	21.6	26.3	3.11	3.8	9.3	36.6	96.2	24.4	25.4	
9	20	2.57	5.6	23.1	89.8	21	24.4	6.89	2.11	4.8	17.2	81.6	22.4	27.9	
10	13	3.47	7.4	31.4	90.4	23.7	25.8	11.6	3.58	7.8	26.7	88.8	26	29.3	

Table 3.5

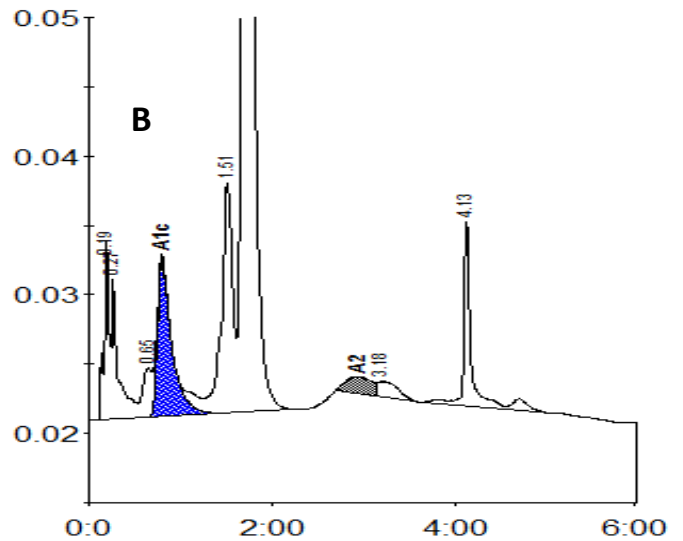
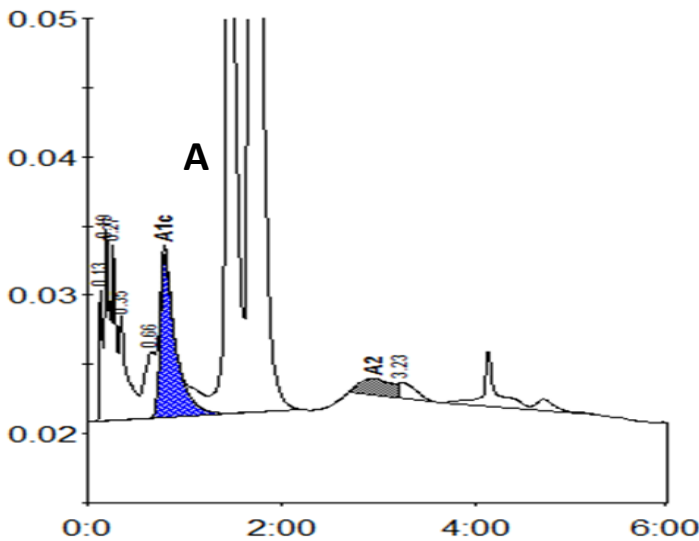
Statistical analysis of pre and post transfusion Wbc, Rbc, Hgb, Hct Mcv, Mch and Mchc parameters in patients transfused with sickle cell trait positive blood using independent t test

Parameter	Pre	Post	t-value	p-value
WBC	12.4±8.6	9.9±6.5	0.744	0.466
RBC	3.6±1.3	3.6±1.0	0.025	0.980
HGB	8.2±2.6	8.5±2.1	0.274	0.787
HCT	28.5±9.3	28.0±6.3	0.146	0.886
MCV	81.7±13.7	81.9±13.2	0.037	0.971
MCH	23.5±3.4	24.5±2.8	0.727	0.476
MCHC	28.2±4.1	29.3±3.8	0.650	0.524

Pre- and post-transfusion High performance liquid chromatography findings among patients transfused with sickle cell trait blood

Post transfusion haemoglobin AS was detected in one of the patients transfused with sickle cell

trait blood as shown in Figure 2B traced to a blood donor who tested positive with sickle cell trait shown in Figure 2C.



Peak	R.time	Height	Area	Area %
Unknown	0.13	9420	20307	0.6
A1a	0.19	13758	51661	1.6
Unknown	0.27	12622	31217	0.9
A1b	0.35	7571	44473	1.3
LA1c/CHb-1	0.66	4806	37797	1.1
A1c	0.80	11755	129552	5.3
P3	1.46	56693	378328	11.4
A0	1.69	488031	2586030	77.9
A2	2.94	1146	26730	2.0
Unknown	3.23	1076	13408	0.4

Peak	R.time	Height	Area	Area %
A1a	0.19	12897	51776	1.7
A1b	0.27	10095	45801	1.5
LA1c/CHb-1	0.65	3570	25054	0.8
A1c	0.80	11399	122149	5.5
P3	1.51	16553	139493	4.6
A0	1.69	487970	2555278	83.9
A2	2.95	1222	25457	2.1
Unknown	3.18	1071	16111	0.5
S-Window	4.13	13294	65587	2.2

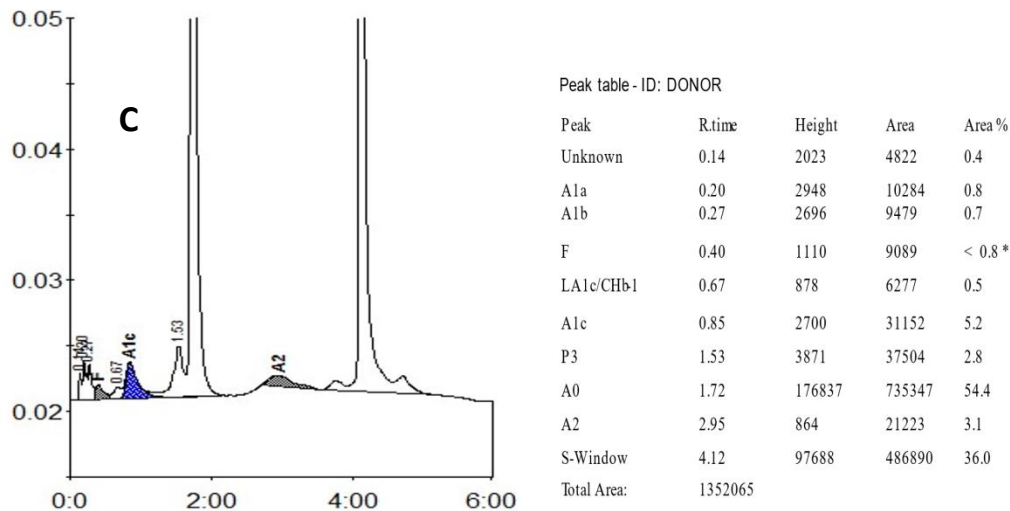


Figure 2A: Pre transfusion chromatogram of a patient with normal haemoglobin AA who was transfused with Sickle cell trait blood.

Figure 2B: Chromatogram of the patient after being transfused with sickle cell trait blood, the concentration of haemoglobin S was 2.2 % as shown in Figure 2B.

Figure 2C: Chromatogram of the sickle cell trait blood donor whose blood was transfused to the above patient, the concentration of haemoglobin S in the blood donor was 36.0 % as shown in Figure 2C.

KEY: Haemoglobin S Window Retention Time 4.12/4.13minutes

Liver function tests for Patients transfused with sickle cell trait blood

None of the ten patients who had been transfused with sickle cell trait blood had elevated bilirubin and transaminases. All the

liver function tests profile were not affected by sickle cell trait transfusions as indicated by the statistical analysis $p= 0.2193, 0.4678, 0.3052,$ and 0.7263 for ALT, AST, DB and TB respectively as shown in Table 3.5 below.

Table 3.5

Statistical analysis of variance between pre and post transfusion liver function profile in patients transfused with sickle cell trait blood

	Pre-transfusion mean	Post-transfusion mean	Pre-transfusion SD	Post-transfusion SD	Pre-transfusion SEM	Post-transfusion SEM	t	df	P value
ALT	6.70	8.30	2.06	3.40	0.65	1.08	1.2729	18	0.2193 ^{ns}
AST	33.80	31.10	7.16	9.01	2.26	2.85	0.7417	18	0.4678 ^{ns}
DB	0.3650	0.3970	0.2291	0.2205	0.0725	0.0697	1.0873	9	0.3052 ^{ns}
TB	0.6640	0.6620	0.2536	0.2495	0.0802	0.0789	0.3612	9	0.7263 ^{ns}

ns= Not Significant

Key ALT: Alanine transferase. AST: Aspartate transferase. DB: Direct bilirubin. TB: Total bilirubin. SD: Standard Deviation. SEM: Standard Error Mean. t: t value df: degrees of freedom

DISCUSSION

Sickle cell trait mainly affects the red blood cells and has been associated with incomplete white cell filtration in donated blood.¹⁵ In this present study, the frequency of sickle cell trait among blood donors in Bungoma County was 14.29%. These findings on Haemoglobin S gene is higher as compared to studies carried out in other countries such as India, which documented sickle cell trait prevalence of 0.08%.¹⁶ However, our findings are lower than the 27.1%, 23.6 % and 19.5 % reported in Nigeria and Democratic republic of Congo respectively.^{7,17} Therefore, variations on the prevalence rates of various haemoglobin variants do occur across the globe and could be attributed to the association of some abnormal haemoglobin to some disease conditions.¹⁸

Apart from the absence of HbAC variants in this study, the HbAA>HbAS haemoglobin pattern (85.71% and 14.29%) a finding that concurs with studies done amongst blood donors in Nigeria which had a similar trend of Hb AA > AS pattern of 72.4% and 27.1% for Hb AA and Hb AS respectively.⁷ The Hb AA > AS haemoglobin sequence reported in this study and other parts of Africa could be attributed to the protective role of Haemoglobin AS towards severe malaria which is prevalent in these regions.¹⁹ This study did not report Hb AC and SC among the blood donation population, this haemoglobin variants have been reported in high frequencies in West Africa and this could be attributed to concept of these genes variations due to different geographical regions and communities.⁵

In this present study red blood cell indices (Red blood cell counts, Haemoglobin, Haematocrit, Mean cell volume, Mean cell haemoglobin and Mean cell haemoglobin concentration) were not statistically significant in patients transfused with sickle cell trait

blood ($p = 0.980, 0.787, 0.886, 0.971, 0.476$ and 0.524 respectively). Total white blood cell counts were also not affected with sickle cell trait blood transfusions ($p = 0.466$). However, it should be noted that such parameters may be affected by transfusing blood with sickle cell trait to patients. For instance, an increase of red blood cell count from 2.50×10^{12} to 3.35×10^{12} per litre, haemoglobin level from 6.8 grams per decilitre to 9.6 grams per decilitre and haematocrit 21.8% to 29.9 % was reported in a diabetic patient who had been transfused with blood containing haemoglobin S in Italy.⁹

The increase in haematological parameters in patients transfused with sickle cell trait blood could be attributed to the normal haematological profile in blood donors with sickle cell trait genes which are similar to sickle cell trait negative blood donors.¹¹ In one study carried out in Nigeria on storage lesions of sickle cell trait donated blood, it did report a reduction in all cell lines and an increase in haemolysis²⁰; However, in this present study, liver function tests were not affected in patients transfused with sickle cell trait blood, Total bilirubin, Direct bilirubin, Alanine transferase, Aspartate transferase ($p = 0.2193, 0.4678, 0.3052$ and 0.7263). These could be attributed to normal haematological profile reported in sickle trait positive blood donors.¹¹ Also another contributing factor could be the fact that these blood units were transfused within one week, sickle blood units stored for a long time have been associated increased haemolysis and reduced cell counts.²⁰

No transfusion reaction was reported in patients exposed to blood with haemoglobin variants (Hb AS) in this present study. This finding concurs with that of a study that was carried out in India where two patients that acquired haemoglobin S at a concentration of 9.9% to 18.5% did not develop any complications.² Also in a study done in

Arkansas Children Hospital in America, 30 patients who had acquired abnormal haemoglobin variants at a range of 1 % to 14.1 % during exchange transfusion did not also develop any complication during or after blood transfusion.¹⁰

However, it should be noted that sometimes adverse reactions can occur during transfusions due to sickle trait blood being transfused to paediatric patients.¹⁰ Fortunately, our findings did not report such findings could be attributed to the adult patients who were the majority in this group and are less vulnerable to sickle cell trait blood as compared to paediatric patients.¹⁰

Despite the rare adverse reactions reported in patients transfused with haemoglobin variants around the world, this study concurs with others which have established the prevalence of abnormal haemoglobin variants in transfused population which leads to misdiagnosis, a factor leading to poor management of patients.

Limitations

The current study assessed Sickle cell trait frequencies in volunteer blood donors only which may not be representative of the whole county population.

CONCLUSION

Sickle cell trait is prevalent among blood donors in Bungoma County. Acquired sickle trait haemoglobin variant is present among transfused population in Bungoma County though adverse reactions have not been reported in any patient transfused with sickle cell trait blood.

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