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Prevalence of Anemia among Adolescent Girls in a Rural Area of Indore, Central India

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Abstract

Background: India is one of the countries with the largest prevalence of anemia. The studies about adolescent anemia limited to small population in rural area around Index Medical College Indore. In this study, we aimed to investigate the prevalence of anemia and risk factors among adolescent girls in a PHC in jurisdiction of this institute, where social customs and diet are applied strictly and adopted as taboo in the society. **Materials and Methods:** The population of study was recruited from 12- to 18-year-old girls from various schools in and around the villages under PHCs having dependent rural population of Index Medical College, Hospital and Research Center, Indore. It also included the adolescent girl patients who applied to Pediatric Outpatient clinics. The criteria of anemia were accepted as the hemoglobin value below 12 g/dl for girls. Thalassemia traits, patients having chronic illness, or taking medication were excluded from the study. The following investigations done were complete blood count, peripheral blood smear study, reticulocyte counts, osmotic fragility test, Sickling test, alkali denaturation test for HbF, and hemoglobin electrophoresis. **Results:** The girls aged 12–18 years were 196 (23.33%) who were selected for the analysis to know the prevalence and type of anemia. The overall prevalence of anemia was 55.10%. About 108 out of 196 adolescent girls had varying severity of anemia. About 44.90% of girls were found to be non-anemic. Out of all 196 adolescent girls, 77 (70.00%) were mildly anemic, 16 (14.54%) were moderately anemia, and 17 (15.45%) were severely anemic. The Mean and SD values found were 11.23 ± 2.53 gm/dl. In the present study, the analysis of the prevalence of morphological types of anemia revealed that 83 (42.34%) girls had normocytic normochromic blood picture. Whereas the microcytic hypochromic peripheral smears were seen in 45 (22.95%) adolescent girls. The microcytic normochromic anemia was found in 50 (25.51%) cases and macrocytic picture was seen in 12 (6.12%) participants. All were advised Hb electrophoresis. Two of the girls did not report and 15 girls' sample were analyzed. It showed 1 (0.51%) girl had sickle cell disease, 5 (2.55%) had sickle cell trait, and nine did reveal for sickle thalassemia disease. **Conclusion:** This study has demonstrated that anemia, among rural girls of small villages and Taluka in Madhya Pradedh, is also high as in other parts of the country. This indicates the importance of including adolescents in the risk group to improve their iron status and the need for planning intervention programs that would increase the hemoglobin levels among adolescent girls through prophylaxis treatment, dietary modification, and helminth control. Increasing the educational level of rural girls would also ensure safe motherhood.

Keywords: Adolescent girls, Anemia, Central India, Rural area

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Introduction

Anemia is one of the most important health problems throughout the world.[1] Worldwide, iron-deficiency anemia is a significant problem and especially in developing countries it is widespread; yet, the most neglected micronutrient deficiency disorder among children, adolescence girls, and pregnant women.[2] Iron-deficiency anemia suppresses human productivity. Although a moderate degree of anemia may not affect every day work, it has massive impact on those engaged in heavy physical activities. The consequences of anemia among women include reduced energy and capacity for work and poor pregnancy outcome that, further, enhances maternal mortality.[2] It is estimated that almost 20% of maternal deaths are directly caused by anemia and causes of another 50% of maternal deaths are associated with anemia in the world.[3]

India is one of the countries with the largest prevalence of anemia. In the past two decades, the importance of iron deficiency and anemia as a public health problem has been increasingly recognized by health authorities and policy makers. This is reflected in the goals on the reduction of iron deficiency anemia (IDA) endorsed by Heads of State, ministers in the World Declaration and Plan of Action from the World Summit for Children (1990), and in the World Declaration and Plan of Action for Nutrition from the International Conference on Nutrition (1992).[4]

Adolescent children are one of the major risk groups for anemia.[5] The prevalence of anemia among adolescents is 27% in developing countries, and 6% in developed countries.[6] In India, the prevalence of anemia at all ages remains very high. It has been reported by ICMR (11 states; 1998–2000) adolescent girls (rural) 84.9% with 9.9% severe anemia.[7] IDA constitutes the major anemia during adolescent period. Accelerated development, hormonal changes, malnutrition, and starting of menstrual periods in girls are major causes in this period.[5,8] Because iron is an essential element for the function of various organs, its deficiency may lead to impaired perception and learning difficulties ending up with declined school success.[9] In childhood, the most frequent

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cause of megaloblastic anemia is the deficiency of Vitamin B12 and folic acid. Fresh vegetables and fruits contain folic acid, but the only source of Vitamin B12 is foods of animal origin.[10] Deficiency of Vitamin B12 may cause neuropsychiatric problems, impaired behavior, lack of attention, learning difficulties, and a decline in the success of the affected students.[11] The prevalence of Vitamin B12 and folic acid deficiency vary among different communities with different eating habits and socioeconomic levels.[12] The studies about adolescent anemia limited to small population in rural area around Index Medical College Indore. In this study, we aimed to investigate the prevalence of anemia and risk factors among adolescent girls in a PHC in jurisdiction of this Institute, where social customs and diet are applied strictly and adopted as taboo in the society.

Aim

The aim of this study was to know the prevalence, cause, and type anemia and also to know the occurrence of hemoglobin disorders in the adolescent girls in the rural population in the villages in surroundings of a tertiary care hospital.

Objectives

The objectives of this study were as follows:

1. To find the prevalence of anemia among all adolescent girls in a cohort of villages
2. To know occurrence of hemoglobinopathies between these adolescent.

Materials and Methods

The population of study was recruited from 12- to 18-year-old girls from various schools in and around the villages under PHCs having dependent rural population of Index Medical College, Hospital and Research Centre, Indore. It also included the adolescent girl patients who applied to pediatric outpatient clinics between December 2011 and October 2013 in Index Medical College, Hospital and Research Center, Indore. This study was approved by the Institutional Ethic Committee. Informed written consent was obtained from legal representatives of patients. The criteria of anemia were accepted as the hemoglobin value below 12 g/dl for girls. Thalassemia traits, patients having chronic illness, or taking medication were excluded from the study.

The patients with anemia were investigated in the Hematology Laboratory at the Department of Pathology. Vitamin B12, folic acid, and ferritin levels were not measured as this was the community-based cross-sectional study. The anemic patients who had peripheral smears suggesting hemolytic anemia were subjected to the cellulose paper hemoglobin electrophoresis by automated electrophoresis device. The following investigations done were complete blood count, peripheral blood smear study, reticulocyte counts, osmotic fragility test, Sickling test, alkali denaturation test for HbF, and hemoglobin electrophoresis.

Cellenium 19 and Sysmex XS 800i fully automated Cell counter were used for CBC. Cellenium 19 is a three-part differential instrument. A three-part differential instrument differentiates the leukocytes into three subpopulations, namely, lymphocytes, granulocytes, and the mid-cell fraction (eosinophils, basophils, monocytes, and precursors of WBCs) by electronic sizing. Specially formulated reagents cause the WBC membrane to shrink around the nucleus while keeping the cell intact, allowing separation of WBCs according to their volume. Sysmex XS 800i is the 5-part differential instruments. It reports on all the five subpopulations, namely, neutrophils, eosinophils, basophils, monocytes, and lymphocytes. MCV is a key diagnostic indicator. An important factor, namely, IDA coexisting with β-thalassemia trait can have very low value for MCV. For all practical purposes, MCV of ≤65 fl indicates possibility of coexistence of β-thalassemia trait and iron deficiency.[13,14]

Red cell distribution width (RDW) is a measure of the degree of variation in red cell size. IDA is characterized by an increase in RDW and thalassemia trait in contrast tends to produce a uniform microcytic red cell population without a concomitant increase in RDW. Therefore, RDW may provide information useful as an adjunct to diagnosis, but it is not useful as alone indicator. RBC counts are a useful diagnostic adjunct, because thalassemia produces ineffective erythropoiesis with microcytichypochromic anemia with an increase in RBC numbers.[15]

Peripheral Blood Smear Study

Principle

The polychromatic staining solution (Wright, Leishman, Giemsa) contains methylene blue and eosin. These basic and acidic dyes induce multiple colors when applied to cells. Methanol acts as a fixative and also as a solvent. The fixative does not allow any further change in the cells and makes them adhere to the glass slide. The basic component of white cells (cytoplasm) is stained by acidic dye and they are described as eosinophilic or acidophilic. The acidic component (nucleus with nucleic acid) takes blue purple shades by the basic dye and they are called as basophilic. The neutral component of the cells is stained by both the dyes.[16]

Staining Method

Smear is covered with the staining solution (Leishman stain). Wait for one minute, then add equal amount of buffer solution. Mix the reaction mixture adequately by blowing on it through a pipette. Wait for 10 min. Wash the smear using tape water (distilled water is not used because sometime it may be slightly acidic). Stand the slide in draining rack for drying.[16] The cells are identified under oil immersion.

Reticulocyte Counts

Principle

Brilliant cresyl blue in an isotonic medium selectively stains nucleic material of erythrocytes called reticulocytes which can be seen under a microscope directly or with a counter stain.[17-19] The reaction takes place only in supravitally stained unfixed preparations.

Osmotic Fragility Test

Principle

The method to be described is based on that of parparteta![20] which is used in this study. The principle of method is a small

volume of blood which is mixed with a large excess of buffered saline solutions of varying concentration. The fraction of red cells lysed at each saline concentration is determined colorimetrically. The test is normally carried out at room temperature (15–25°C).

Sickling Test

When red cells containing Hb S are subjected to deoxygenation, they become sickle-shaped while cells that do not contain Hb S remain normal. Certain reducing chemical agents such as 2% sodium metabisulfite or sodium dithionite can deprive red cells of oxygen. Blood and 2% sodium metabisulfite reducing agent in equal proportion was mixed on a glass slide and a cover slip was placed over it; then, it was sealed with petroleum jelly or paraffin wax mixture. Amount of HbS in red cells and degree of deoxygenation influence the speed and extent of sickling. Sickling is usually evident after 30 min; if it is not then the slide is re-examined after allowing it to stand overnight. The sickled cells have minimum of two pointed projections.[21]

Alkali Denaturation Test for HbF

HbF may be estimated by several method based on its resistance to denaturation at alkaline pH, by HPLC or by an immunological method.[22] The alkaline denaturation methods of Betke *et al*. [23] is reliable for small amounts (<10–15%) of Hb F; whereas for levels of more than 50% and in cord blood, the method of Jonxis and Visser *et al*. [24] is preferable. However, this method is not reliable at levels of <10%.

Hemoglobin Electrophoresis

Cellulose acetate electrophoresis at alkaline pH

Hemoglobin electrophoresis at pH 8.4–8.6 using cellulose acetate membrane is simple, reliable, and rapid. It is satisfactory for the detection of most common clinically important hemoglobin variants.[25-27]

Principle

At alkaline pH, hemoglobin is a negatively charged protein and when subjected to electrophoresis will migrate toward the anode (+). Structural variants that have a change in the charge on the surface of the molecule at alkaline pH will separate from Hb A. Hemoglobin variants that have an amino acid substitution that is internally sited may not separate and those that have an amino acid substitution that has no effect on overall charge will not separate by electrophoresis.[26,27] Interlab master kit contain staining and destaining solution, clearing solution, and buffer for migration chamber. Four sample cellulose acetate strips were used in this apparatus. In this study, the hemoglobinopathies were detected by fully automated electrophoretic apparatus of Interlab GenioS. Interlab master kit of reagents is used in this apparatus.

Results

The present study was a cross-sectional study conducted at villages under Vantamuri PHC having dependent rural population of Index Medical College, Hospital and Research Center, Indore. The study included 840 all school going girls of all ages, attending primary, middle and high (Junior Colleges) school. The girls aged 12–18 years were 196 (23.33%) who were selected for the analysis to know the prevalence and type of anemia. The pediatric aged 12–14 years and adolescence was elder than 14 years up to 18 years of age were the main target for this study. We analyzed 108 participants above 12 years to 18 years. In this study, we considered hemoglobin of 12 gm/dl and below as anemia.

Majority of the girls were in the age group of 15–18 years. There were no participants in the age group of 19 years [Table 1].

The overall prevalence of anemia was 55.10%. About 108 out of 196 adolescent girls had varying severity of anemia. About 44.90% of girls were found to be non-anemic. It is well established that most of the adolescent girls suffer from IDA or may have both IDA with folic acid and B12 deficiency [Table 2 and Figure 1].

We classified anemia as mild <12 g/dl to 10 g/dl, moderate <10 g/dl to 08 gm/dl, and severe as <8 g/dl. Out of all 196 adolescent girls, 77 (70.00%) were mildly anemic, 16 (14.54%) were moderately anemia, and 17 (15.45%) were severely anemic. The various parameters found varied from low to high be analyzed and are depicted as well. Hemoglobin was as low as 1.00 g/dl to high as 14.9 g/dl. The Mean and SD values found were 11.23 ± 2.53 g/dl. We also analyzed RDW% in all the 196 cases which varied from 7.5% to 27.9%. The value was between 7.5% and 12.5% which were

Table 1: Distribution of study participants according to the girls' age

Age (in years)	*Study participants*	
	Number	*Percentage*
12	15	7.65
13	18	9.18
14	33	16.84
15	36	18.37
16	43	21.94
17	25	12.76
18	26	13.27
Total	196	100

Table 2: Distribution of study participants in relation to anemia

Anemia	*Study participants*	
	Number	*Percentage*
Anemia	110	55.10
Non-anemia	86	44.90
Total	196	100

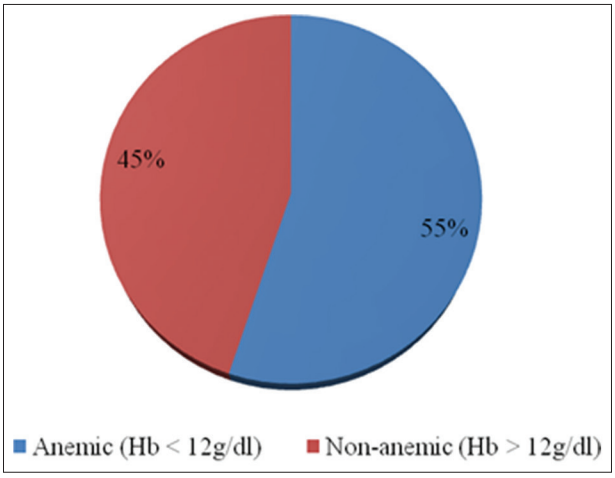

Figure 1: Distribution of study participants in relation to anemia

considered as normal distribution of the red cells and value above 12.5% was abnormal. In our study, most of the anemic girls (56) showed abnormal RDW %, whereas the girls with normal hemoglobin (123) and with hemoglobinopathies (17) had RDW% value below 12.5% within normal range [Tables 2-4 and Figure 2].

In the present study, the analysis of the prevalence of morphological types of anemia revealed that 83 (42.34%) girls had normocytic normochromic blood picture. Whereas the microcytic hypochromic peripheral smears were seen in 45 (22.95%) adolescent girls, the microcytic normochromic anemia was found in 50 (25.51%) cases and macrocytic picture was seen in 12 (6.12%) participants [Table 5].

Table 6 is showed that the best mean Hb ranged as 10.80 in girls at the age of 12 years as they attain age of menarche. However, they show a fall in the Hb level as they grow up to 14–18 years of their age. This is attributed to regular loss of blood with low nutritional elements in diet particularly the Iron content.

Out of 196 girls, 66 (33.67%) were in the age group of 10–14 years, where 130 (66.33%) were in the age group of 15–18 years. Thus, majority of girls were in the age group of 15–18 years [Table 7].

The above Table 8 depicts the prevalence of hemoglobinopathy in the study. Out of 196 girls, 17 adolescent girls were suspicious of hemoglobin disorder on CBC and peripheral blood smear examination. All were advised Hb electrophoresis. Two of the girls did not report and 15 girls' sample were analyzed. It showed 01 (0.51%) girl had sickle cell disease, 5 (2.55%) had sickle cell trait, and nine did reveal for sickle thalassemia disease.

Observations

The prevalence of anemia in was studied by our rural tertiary health-care center as a community health program among the adolescent girls (12–18 years) representing 14 subcenter villages of block of Indore and Dewas districts of Madhya Pradesh. In this study, we included 840 school going girls. Out of these, 196 (23.33%) were the adolescent girls. We found the prevalence of anemia in 110 (55.10%) girls. The anemia was considered to be present if Hb was <12 gm/dl.[28] The prevalence of mild, moderate, and severe anemia among adolescent girls was 70.0%, 14.54%, and15.45%, respectively.

Majority were having mild anemia and only 15.45% severe anemia [Table 3]. Anemia was found to be significantly associated with educational and socioeconomic status of parents. It was also well correlated with age, anthropometry, and menarcheal age of the girl child by Rawat *et al*.[29]

Adolescence is a crucial phase of growth in the life cycle of an individual. It is a period of transition between childhood and adulthood occurring between 12 and 18 years of age.[30] Adolescents of both the sexes are particularly vulnerable to developing anemia due to rapid growth, weight gain, and blood volume expansion and in girls additionally due to onset of menstruation. In girls, middle adolescence growth happens earlier (i.e., during 12–15 years) than in boys (i.e., during 13–16 years). Adolescent girls form a crucial segment of the population and constitute, as it were**,** the vital "bridge" between the present generation and the next.[30]

We found that the rate of anemia was as high as 55.10% in adolescent school going girls. The ICMR has reported by ICMR (1998–2000) that in adolescent girls' population in India is the most vulnerable next to pregnant females. In one of the task force study, they found that the adolescent children are one of the major risk groups for anemia.[31] The prevalence of anemia among adolescents is 27% in developing countries, and 6% in developed countries. In India, the prevalence of anemia at all ages remains very high. It has been found adolescent girls (rural) 84.9% with 9.9% severe anemia.[31] Our results were comparable with the average rate of anemia in the Indian population. In adolescence period, the iron need is increased due to rapid growth. To increase the absorption of iron, the level of ferritin decreases. In addition, the onset of menstruation in girls results in reduced ferritin levels. The prevalence of anemia was 44.8% with severe anemia being 2.1%, moderate 6.3%, and mild anemia 36.5%. There was a decrease in

Table 3: Distribution of study participants in relation to the severity of the anemia

Degree of anemia	*Anemic Study participants (n=110)*	
	Number	*Percentage*
Mild anemia	77	70.0
Moderate anemia	16	14.54
Severe anemia	17	15.45
Total	110	100.0

Table 4: Distribution of study participants in relation to the hematological parameters

Variable	*Mean±SD*	*Min-Max*
Hemoglobin (g/dL)	11.23±2.53	1.00–14.90
MCV (fl)	81.77±11.88	52.30–108.00
RDW (%)	14.85±4.22	7.5–27.9

Table 5: Distribution of study participants according to the type of peripheral blood picture

	Number (n=196)	*Percentage*
Macrocytic normochromic blood picture	12	6.12
Microcytic normochromic RBCs	50	25.51
Normocytic normochromic blood picture	83	42.34
Microcytic hypochromic	45	22.95
Microcytic normochromic	3	1.53
Microcytic severely anemic	1	0.51
Normocytic hypochromic	1	0.51
Anisocytosis and hypochromasia	1	0.51
Total	196	100.0

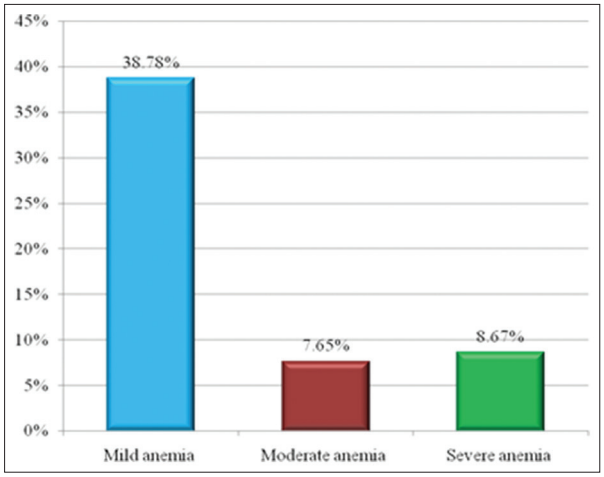

Figure 2: Distribution of study participants in relation to the severity of the anima

Table 6: Distribution of study participants according to their age and anemia

Age (in years)	*Total*	*Anemic*		*Mean Hb*
	Number	*Number*	*Percentage*	
12	15	6	5.45	10.80
13	18	11	10.00	10.03
14	33	20	18.18	9.80
15	36	19	17.27	10.37
16	43	21	19.09	9.51
17	25	14	12.73	10.03
18	26	19	17.27	10.19

Table 7: Distribution of study participants as early and late adolescents

Age (in years)	*Study participants*	
	Number	*Percentage*
10-14	66	33.67%
15-18	130	66.33%
Total	196	100%

Table 8: Demographic information

Total adolescent girls during study period	196
Total number of patients with sickle cell disease	01
Incidence of sickle cell disease in girls	0.51%
Total number of patients with sickle cell trait	02.55%
Total number of thalassemia patients	0
Incidence of thalassemia	0.00%
Total number of sickle cell β-thalassemia patients	09
Incidence of sickle cell β-thalassemia patients	4.59%

the prevalence as the age increased; however, the difference was not statistically significant. The prevalence of anemia was 40.7% in premenarcheal girls as compared to 45.2% in post menarcheal girls. In our study, we found that 99 (90%) girls were anemic in comparison to 11 (10%) non-anemic in menstruating adolescent girls.[32]

In one study, it has been documented that the prevalence of anemia was 46.6% in premenarcheal girls as compared to 48.4% in postmenarcheal girls in the urban slums of North East Delhi.[33] Vasanthi *et al*.[34] observed a prevalence of 27% and 22% in the rural and urban premenarcheal girls and 24.2% and 27.8% in the rural and urban postmenarcheal girls in the age group 11–16 years, respectively, in Hyderabad. In the present study, we found this was 40.7% premenstrual girls and 45.2%, in postmenstrual girls. Irregular eating habits and the lower consumption of animal source foods contribute to the development of anemia in this population of society. Therefore, girls have higher incidence of anemia. In the present study, 70% of anemic subjects had low MCV with high RDW, suggestive of probably iron deficiency.[30]

In our study out all 840 participants, the adolescent girls were 196. Out of these, 110 (56.10%) were detected to have anemia of varying degree as no anemia 86 (44.90%), mild anemia 77 (70%), moderate anemia 16 (14.54%), and severe anemia 17 (15.46%). The study revealed that the overall prevalence of anemia as 55.10% in school going adolescent girls which are in accordance with Kapoor *et al*.[35] (60%), Singh *et al*.[36] (56%), Rana *et al*.[37] (60%), and Seshadri *et al*.[38] (63%). However, Chaturvedi *et al*.,[39] Kotecha *et al*.,[40] and Agarwal[41] reported a higher prevalence of 73.7%, 74.7%, and 47.6%, respectively.[42]

The prevalence of anemia is very high among all the study population in India. The results show that more than 95% of children, adolescent girls, and pregnant women suffer from anemia. The prevalence is the highest among the adolescents, 97.8%. Nearly half of the children are mildly anemic and 2.9% are severely anemic. More than half of the pregnant women in the country are mildly anemic and 42.6% are moderately anemic. The situation is the worst for adolescent girls, 27.1 % of whom are severely anemic.

There are so many disorders of red blood cells, but hemoglobinopathy is most common disorder. Most common hemoglobinopathy in this study was sickle cell β-thalassemia (4.59%), sickle cell trait (2.55%), and followed sickle cell disease (0.51%). The prevalence of β-thalassemia major and thalassemia trait alone was nil. All their families were given genetic counseling. These findings could not be at all correlated as this has a very small select group, not representing true reflection of that population. Therefore, the findings revealed could not be extrapolated. In one study done in the same area showed, maximum prevalence of 69.38% in the same geo-socio population.[43]

Limitations

The study was confined among the selected groups; though it is recognized that adolescent girls in the society have high risk of anemia due to various factors including their nutritional needs particularly at the menarche as periodic loss of blood needs nutrients in form of iron and folic acid in their diet, as anemia limits their social and physical activities.

Conclusion

This study has demonstrated that anemia, among rural girls of small villages and taluka in Madhya Pradesh, is also high as in other parts of the country. This indicates the importance of including adolescents in the risk group to improve their iron status and the need for planning intervention programs that would increase the hemoglobin levels among adolescent girls through prophylaxis treatment, dietary modification, and helminth control. Increasing the educational level of rural girls would also ensure safe motherhood.

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