# The Frequency of Iron Deficiency Anemia and Thalassemia Trait among Children: Experience at Prince Rashed Bin Al-Hassan Military Hospital

*Zuhair Nusair MD*, Abdelrazzaq Al-Wraikat MD**, Nazih Abu Al-Shiekh MD**, Sameer Kofahi MD^, Mohammad Zoubi MD^*

## ABSTRACT

**Objectives**: To determine the frequency of iron deficiency anemia and thalassemia trait among children attending the Pediatric Department at Prince Rashed Bin Al-Hassan Military Hospital in the North of Jordan.

**Methods:** This hospital based study was conducted in the year 2008 on 1,012 children aged 6 months to 14 years who attended the Pediatric Department at Prince Rashed Bin Al-Hassan Military Hospital in North of Jordan using fully automated blood cell counter for of the mean corpuscular volume, serum ferritin level and high performance liquid chromography, or genotyping. None of the subjects included in the study had been on any hematinic in the previous six months, had infection in the past one month or had a chronic disease. The diagnosis of iron deficiency anemia was defined as mean corpuscular volume ≤ mean – 1 standard deviation corrected for age, with a ferritin level < 7 ng/ml of the serum (normal reference range 7 – 140 ng/ml). The diagnosis of thalassemia trait, for subjects with normal or high serum ferritin and those whose mean corpuscular volume was non-compliant to iron therapy, was obtained by high performance liquid chromography or polymerase chain reaction, which was performed at Princess Eman Research and Laboratory Science Center.

**Results:** The frequency of iron deficiency anemia and thalassemia trait was 13.3% and 5.8% respectively. They were equally frequent among males and females. The age specific-rate was as follows: 6 months to 2 years 7.4% iron deficiency anemia and 1.3% thalassemia trait, 2 - 6 years 3.1% iron deficiency anemia and 2.5% thalassemia trait, 6 - 12 years 1.6% iron deficiency anemia and 1.2% thalassemia trait and 12-14 years 1.3% iron deficiency anemia and 0.9% thalassemia trait.

**Conclusion**: The frequency of iron deficiency anemia (13.3%) and thalassemia trait (5.8%) among children in North of Jordan was estimated by statistical measurement of mean corpuscular volume fL. It is a strong cost effective predictor in the majority of cases. The red-blood indices complemented with serum ferritin and high performance liquid or polymerase chain reaction are of value for precise diagnosis.

**Key words:** Children, Frequency, Iron deficiency anemia, North of Jordan, Thalassemia Trait

**JRMS March 2011; 18(1): 39-44**

## Introduction

Anemia is a major pediatric health problem and iron deficiency anemia (IDA) is the most common cause of anemia,[1-3] it is usually due to nutritional

From the Departments of:
* Pediatrics, Prince Rashid Bin Al-Hassan Hospital, (PRHH), Irbid-Jordan
** Princess Iman Research and Laboratory Sciences Center, King Hussein Medical Center (KHMC), Amman-Jordan
^ Preventive Medicine, (KHMC)
Correspondence should be addressed to Dr. Z. Nusair, (PRHH), Irbid-Jordan, E-mail: zuhair1963@yahoo.com
Manuscript received April 9, 2009. Accepted June 18, 2009

imbalances in children among vulnerable groups of population in Jordan, and it is an important public health problem worldwide.[4-8]

Iron deficiency is not normally present in hereditary anemias like thalassemias,[9] but it is one of the causes of microcytic hypochromic anemia. Moreover, in many parts of the world, thalassemia trait (TT) as a cause of microcytic anemia is only less prevalent than IDA and is sometimes confused with it, since it is characterized by microcytosis and sometimes mild anemia. The use of mean corpuscular volume (MCV) to classify anemias as microcytic, normocytic or macrocytic is a standard diagnostic approach.[4,9] On other hand the red-cell distribution width coefficient variation percentage (RDW-CV%) has been reported to be of value in the discrimination of IDA from TT.[10-12] It is an index of the variation in the size of red cells and can be used to detect subtle degrees of anisocytosis.[13] Therefore, an elevated RDW-CV% appears to be the earliest hematolological manifestation of IDA.[14-16]

The serum ferritin concentration is particularly informative in estimating the amount of iron storage and provides direct information about any deficit in the iron nutritional status. Moreover, it is the first in line to drop if the individual suffers any iron deficiency from diet as seen in IDA.[17-19] On other hand, the serum ferritin level, which is usually conducted in conjunction with MCV, can indirectly help understand iron metabolism as MCV measures how large the red blood cells are. When a deficiency in iron occurs, not enough hemoglobin is made. This leads to a reduced rate of red blood cell production and the red blood cells become smaller (microcytic) and paler (hypochromic) than normal.[3,20,21]

The measurement of hemoglobin $A_2$ ($HbA_2$) and hemoglobin F (HbF) by High performance liquid chromography (HPLC) is rapid, reproducible and an appropriate method for screening ß-thalassemia carriers,[22] which have been characterized by microcytosis, hypochromia and $HbA_2$ above 3.5%.[23] On the other hand, the polymerase chain reaction (PCR) is used for the diagnosis of a few number of α-thalassemia trait subjects that should be considered in all children of high risk family origins with a blood picture suggestive of ß-thalassemia trait but in whom the level of $HbA_2$ and HbF are within normal limits.[4]

The aim of this study was to determine the frequency of IDA and TT among children attending the Pediatric Department at Prince Rashed Bin Al-Hassan Military Hospital in the North of Jordan based on MCV, serum ferritin level and HPLC or PCR.

## Methods

This study was conducted in the period between January and December 2008, on all children who were seen at the out patient clinic of the Pediatric Department of Prince Rashed Bin Al-Hassan Military Hospital in the North of Jordan. The study subjects included those whom aged is between 6 months to 14-years-old, not on any hematinic in the previous six months, had no infection in the past one month and had no chronic diseases. The Jordanian Royal Medical Services Ethics Committee approved the study. All subjects had a complete blood count and RBC indices performed at the first visit during the study period.

The cutoff value for the diagnosis of microcytic anemia was arbitrarily set at MCV $\leq$ mean $-$ 1 standard deviation (1SD) of the corresponding age group. If a subject was found to have an MCV below the cutoff value, serum ferritin level was estimated and if it was found < 7 ng/ml (normal reference range 7 - 140 ng/ml) a provisional diagnosis of IDA was made and iron replacement therapy was started. RBC indices were repeated monthly and after 3 months of iron replacement therapy if the MCV improved and became above the cutoff value, the diagnosis of IDA was considered confirmed. If the MCV did not improve and remained below the cutoff value, HPLC was done to confirm or exclude β-thalassemia. If β-thalassemia was excluded, PCR was performed to confirm or exclude α-thalassemia.

If serum ferritin level was within the normal range, a diagnosis of "possible thalassemia" was made and HPLC was done to confirm or exclude β-thalassemia. If β-thalassemia was excluded, PCR was done to confirm or exclude α-thalassemia. HPLC and PCR for all subjects in this study were performed at Princess Eman Research and Laboratory Science Center.

Demographic data included the age, and gender in addition to RBC indices, serum ferritin, and the results of HPLC and/or PCR were recorded and analyzed using Statistical Package of Social Science (SPSS) software. Fisher exact test was used to compare the level of serum ferritin and RBC indices between males and females, RBC indices between different age categories and to compare serum ferritin level and RBC indices between non-anemic, IDA, and TT subjects. Descriptive statistics and

**Table I.** Comparison of the RBC indices (n = 1012) and serum ferritin (n = 227) between males and females

| Parameter | Males | Females | P-Value |
|---|---|---|---|
| Hematocrit or PCV (%) | 34.6 ± 3.9 | 34.3 ± 3.8 | 0.540 |
| MCV fL | 74.5 ± 6.5 | 75.4 ± 6.8 | 0.051 |
| MCH pg | 23.9 ± 2.7 | 24.2 ± 2.9 | 0.057 |
| RDW-CV% | 14.5 ± 2.3 | 14.2 ± 2.4 | 0.077 |
| RBC x $10^{12}$/L | 4.68 ± 0.59 | 4.63 ± 0.51 | 0.140 |
| Serum ferritin | 12.9 ± 14.5 | 13.1 ± 15.2 | 0.891 |

**Table II.** Age-Specific RBC indices

| Age group | No. | PCV | MCV | MCH | RDW-CV | RBC |
|---|---|---|---|---|---|---|
| 6 mo to< 2 yr | 403 | 33.4 ± 3.5 | 72.8 ± 6.2 | 22.9 ± 2.6 | 15.2 ± 2.6 | 4.69 ± 0.49 |
| 2 to< 6 yrs | 329 | 34.3 ± 3.3 | 75.2 ± 6.2 | 24.3 ± 2.6 | 14.1 ± 2.0 | 4.62 ± 0.64 |
| 6 to< 12 yrs | 201 | 35.8 ± 4.2 | 78.0 ± 5.8 | 25.5 ± 2.4 | 13.4 ± 1.6 | 4.59 ± 0.48 |
| 12-14 yrs | 79 | 36.7 ± 4.6 | 76.8 ± 8.6 | 24.6 ± 3.6 | 14.1 ± 2.4 | 4.79 ± 0.61 |
| Overall | 1012 | 34.4 ± 3.8 | 74.9 ± 6.6 | 24.0 ± 2.8 | 14.4 ± 2.3 | 4.66 ± 0.56 |

**Table III.** Age-specific frequency of IDA and TT

| Age group | Non-anemic No. (%) | IDA No (%) | TT No (%) |
|---|---|---|---|
| 6 mo - < 2 yr | 315 (31.1) | 75 (7.4) | 13 (1.3) |
| 2 - < 6 yrs | 273 (27.0) | 31 (3.1) | 25 (2.5) |
| 6 - <12 yrs | 173 (17.1) | 16 (1.6) | 12 (1.2) |
| 12 - <14 yrs | 57 (5.6) | 13 (1.3) | 9 (0.9) |
| Total | 818 (80.8) | 135 (13.3) | 59 (5.8) |

**Table IV.** Gender-specific frequency of IDA and TT

| Age | Non-anemic No (%) | IDA No (%) | TT No (%) |
|---|---|---|---|
| Males | 436 (43.1) | 83 (8.2) | 35 (3.6) |
| Females | 382 (37.7) | 52 (5.1) | 24 (2.4) |
| Total | 818 (80.8) | 135 (13.3) | 59 (5.8) |
| P-value | | 0.054 | 0.520 |

**Table V.** Comparison of the serum ferritin (n = 227) and RBC indices (n = 1012) between IDA, and TT.

| Parameter | Non-anemic | IDA | TT | P-value |
|---|---|---|---|---|
| Serum ferritin ng/ml | 15.9 ± 3.9 | 4.3 ± 1.9 | 28.6 ± 18.6 | < 0.0001 |
| Hematocrit or PCV (%) | 34.9 ± 3.4 | 32.2 ± 4.8 | 31.9 ± 3.5 | 0.721 |
| MCV fL | 76.9 ± 4.6 | 67.9 ± 6.9 | 62.4 ± 4.7 | < 0.0001 |
| MCH pg | 24.9 ± 1.9 | 20.6 ± 3.0 | 19.3 ± 1.9 | 0.003 |
| RDW CV% | 13.6 ± 1.3 | 18.5 ± 2.4 | 16.3 ± 2.4 | < 0.0001 |
| RBC x $10^{12}$/L | 4.56 ± 0.46 | 4.97 ± 0.69 | 5.22 ± 0.74 | < 0.023 |

cross-tabulation were used to find the frequencies of IDA and TT corrected for age, and the overall frequency of IDA and TT. The Mann-Whitney U test was used to compare between males and females in the rates of IDA and TT, and to see if IDA and TT show sex predilection compared to the non-anemic reference group.

## Results

During the study period, 1,012 children fulfilled the inclusion criteria. They were 554 males and 458 females. Of these, 227 (22.43% of the total sample) had an MCV below the cutoff value for this study. They were 135 (59.5%) males, 92 (40.5%) females. 33 out of the microcytic subjects come for follow up appointment and the MCV was somewhat improved, they included in a non-anemic group because of normal serum ferritin, in addition HPLC and PCR excluded any evidence of a β or α-thalassemia. The remaining 194 microcytic subjects in this study presented as either IDA or TT.

There were no statistically significant differences in RBC indices and serum ferritin level between males and females (P-value > 0.05), as shown in Table I.

Table II, shows the PCV percentage, MCV (fL) and MCH (pg) to be directly related to the age of the subjects (increased with increasing age), while RDW-CV% was indirectly related to the age of subjects (decreased as age increased).

The overall frequency of IDA and TT among children in the current study was 135 (13.3%) and 59 (5.8%) respectively. Of the 59 thalassemic subjects 57 (96.6%) were β-thalassemic and 2 (3.4%) were α-thalassemic. It is interesting however that the 2 α-thalassemic subjects had concomitant iron deficiency proven by low serum ferritin level.

The frequency of IDA and TT among different age groups was as follows: 6 months to < 2 years (7.4% IDA and 1.3% TT), 2 to < 6 years (3.1% IDA and 2.5% TT), 6 to < 12 years (1.6% IDA and 1.2% TT) and 12-14 years (1.3% IDA and 0.9% TT). The highest rate of IDA was in the age group 6 months – < 2 years as demonstrated in Table III.

Table IV, illustrated that the rates of IDA and TT were similar among males and females (P-value > 0.05), as follows: males (8.2% IDA and 3.6% TT) and females (5.1% IDA and 2.4% TT).

The MCV fL, MCH pg and RDW-CV% were significantly higher among IDA subjects than TT subjects, 67.9 ± 6.9fL versus 62.4 ± 4.7 fL, 20.6 ± 3.0 pg versus 19.3 ± 1.9 pg and 18.5 ± 2.4% versus 16.3 ± 2.4%, respectively. while serum ferritin and red blood cell count were significantly higher among TT subjects than IDA subjects, 28.6 ± 18.6 versus 4.3 ± 1.9 ng/ml and 5.22 ± 0.74 versus 4.97 ± 0.69, respectively (P-value < 0.05). Hematocrit on the other hand was not statistically significantly different between the two groups (32.2 ± 4.8 % for IDA and 31.9 ± 3.5% for TT) (P-value = 0.721) as shown in Table V.

## Discussion

In this study, 22.43% of children who were seen in the Pediatric Department of Prince Rashed Bin Al-Hassan Military Hospital in the North of Jordan had microcytic anemia. Of these, the overall rate of IDA and TT was identified as 13.3% and 5.8% respectively. Previous studies demonstrated a prevalence of 5.4% of IDA among schoolchildren in north-eastern Badia in Jordan,[24] other studies reported a prevalence of ß-thalassemia trait and α-thalassemia trait in the north of Jordan of 3.1% and 1% respectively.[23,25] On the other hand, the prevalence of IDA in the United States has been reported to range from 3% to 10% and may be as high as 30% in low-income population.[4,26]

The current study showed that the highest rate of IDA was in the age group of 6 months to <2 years which is consistent with a study that reported the prevalence of IDA among Kelantanese children aged 8 -26 months in Malaysia as high as 31.6%. This could be attributed to prolonged breastfeeding beyond six months of age and failure to introduce formula milk at later infancy.[27]

In this study, infants below the age of 6 months were excluded because at this age they have high stores of iron, which are unlikely to be depleted, although breast milk is low in iron content.[26] Therefore, iron supplementation or fortification of food is recommended.[28,29]

The current study showed that the rate of IDA and TT was not related to the sex of the patient. These findings are consistent with those of studies that reported the prevalence of IDA among schoolchildren of different socio-economic status in urban Turkey[30] and in children aged 6-60 months in Thail and[31] Thalassemia trait on the other hand is a group of autosomal-recessive inherited human disorders, resulting from defects in hemoglobin synthesis and is known to affect males and females equally.[23,32-35]

This study also revealed that the MCV (fL) was lower among TT patients and the RDW-CV% was higher among IDA patients. This could be attributed to the fact that RDW-CV% is a measure of the degree of variation in red cell size. Some causes of microcytic anemia, most notably IDA, are characterized by an increased RDW-CV%. The thalassemias in contrast, tend to produce a uniform microcytic red cell population without a concomitant increase in RDW-CV%.[11,3-16,26,36-38]

Limitations of the present study include the restricted sampling of children who attended the department where the study was conducted and therefore may not represent the whole population. Moreover, we did not study the socio-economic, demographic variables, feeding practices and racial differences attributed to underlying cause of microcytic anemia. The investigations by serum ferritin and hemoglobin electrophoresis or PCR were performed for children with blood picture of microcytosis. Therefore, it is not possible to detect all subjects with TT by screening on the basis of the full blood count as some subjects have normal RBC indices (silent TT). We did not use the hemoglobin level to define the severity of IDA. However, our study was carried out for the purpose of the best estimation for the frequency of IDA, and TT in limited samples North of Jordan regardless of the types of TT and severity of IDA, and it must have detected the great majority of cases.

## Conclusion

The frequency of IDA (13.3%) and TT (5.8%) among children in North of Jordan was estimated by statistical measurement of MCV fL, is a cost effective predictor in the majority of cases. The red-blood indices complemented with serum ferritin and HPLC or PCR are of value for precise diagnosis.

## References


1. **Willows ND, Morel J, Gray-Donald K.** Prevalence of anemia among James Bay Cree infants of northern Quebec. *CMAJ* 2000 Feb 8; 162(3): 323-326.
2. **Earley A, Valman HB, Altman DG, *et al.*** Iron deficiency, and thalassaemia in preschool children. *Arch Dis Child* 1990 Jun; 65(6): 610-614.
3. **Jayaranee S**, **Sthaneshwar P.** Serum soluble transferrin receptor in hypochromic microcytic anaemia. *Singapore Med J* 2006 Feb; 47(2):138-142.
4. **Irwin JJ, Kirchner JT.** Anemia in children. *Am Fam Physician* 2001 Oct 15; 64(8):1379-1386.
5. **Pinsk V, Levy J, Moser A, *et al.*** Efficacy and safety of intravenous iron sucrose therapy in a group of children with iron deficiency anemia. *Isr Med Assoc J* 2008 May; 10(5):335-338.
6. **Shibukawa AF, Silva EM, Ichiki WA, *et al.*** Prophylaxis for iron deficiency anemia using ferrous sulfate among infants followed up at a primary healthcare unit in the municipality of Embu-SP (2003/2004). *Sao Paulo Med J* 2008 Mar 6; 126(2):96-101.
7. **Gong YH, Ji CY, Zheng XX, *et al.*** Correlation of 4-month infant feeding modes with their growth and iron status in Beijing. *Chin Med J (Engl)* 2008 Mar 5; 121(5):392-398.
8. **Faqih AM, Kakish SB, Izzat M.** Effectiveness of intermittent iron treatment of two- to six-year-old Jordanian children with iron-deficiency anemia. *Food Nutr Bull* 2006 Sep; 27(3):220-227.
9. **Mohanty D, Mukherjee MB, Colah RB, *et al.*** Iron deficiency anaemia in sickle cell disorders in India. *Indian J Med Res* 2008 Apr; 127(4):366-369.
10. **Richardson M.** Microcytic anemia. *Pediatr Rev* 2007 Jan; 28(1):5-14.
11. **Clarke GM, Higgins TN.** Laboratory investigation of hemoglobinopathies and thalassemias: review and update. *Clin Chem* 2000 Aug; 46(8 Pt 2):1284-1290.
12. **Jimenez CV.** Iron-deficiency anemia and thalassemia trait differentiated by simple hematological tests and serum iron concentrations. *Clin Chem* 1993 Nov; 39(11 Pt 1):2271-2275.
13. **Oski FA**. Iron deficiency in infancy and childhood. *N Engl J Med* 1993 July 15; 329:190-193.
14. **Melo MR, Purini MC, Cancado RD, *et al.*** The use of erythrocyte (RBC) indices in the differential diagnosis of microcytic anemias: is it an approach to be adopted. *Rev Assoc Med Bras* 2002 Jul-Sep; 48(3): 222-224.
15. **Quadri MI, Islam SM, Nasserullah Z.** The Effect of α-thalassemia on cord blood red cell indices and interaction with sickle cell gene. *Ann Saudi Med* 2000; 20(5-6):367-371.
16. **Dugdale AE**. Predicting iron and folate deficiency anaemias from standard blood testing: the mechanism and implications for clinical medicine and public health in developing countries. *Theor Biol Med Model* 2006 Oct 9; 3:34.
17. **Ragab L, Ibrahim HA, Eid AS, *et al.*** Suitability of soluble transferrin receptor for the clinical diagnosis of different types of anaemia in children. *East Mediterr Health J* 2002 Mar-May; 8(2-3):298-307.
18. **Metallinos-Katsaras E, Valassi-Adam E, Dewey KG, *et al.*** Effect of iron supplementation on cognition in Greek preschoolers. *Eur J Clin Nutr* 2004 Nov;58(11):1532-1542.
19. **Olivares M, Walter T, Cook JD, *et al***. Usefulness of serum transferrin receptor and serum ferritin in diagnosis of iron deficiency in infancy. *Am J Clin Nutr* 2000 Nov; 72(5):1191-1195.
20. **Kotru M, Rusia U, Sikka M, *et al***. Evaluation of serum ferritin in screening for iron deficiency in tuberculosis. *Ann Hematol* 2004 Feb; 83(2):95-100.
21. **Cook JD**. Diagnosis and management of iron-deficiency anaemia. *Best Pract Res Clin Haematol* 2005 Jun; 18(2):319-322.
22. **Tan GB, Aw TC, Dunstan RA, *et al***. Evaluation of high performance liquid chromatography for routine estimation of hemoglobin's A2 and F. *J Clin Pathol* 1993 Sep; 46(9):852-856.
23. **Gharaibeh NS, Al-Sheyyab M, Batieha A**. Detection of β-Thalassemia Carrier in Jordan. *Ann Saudi Med* 2006 Jan-Feb; 18(4):360-362.
24. **Babiker MM, Bashir N, Sarsour N.** Prevalence of thalassaemia in schoolchildren in north-eastern Badia, Jordan. *East Mediterr Health J* 1999 Nov; 5(6):1165-1170.
25. **Bashir N, Barkawi M, Sharif L, *et al***. Prevalence of hemoglobinopathies in north Jordan. *Trop Geogr Med* 1992 Jan; 44(1-2):122-125.
26. **Kazal LA Jr**. Prevention of iron deficiency in infants and toddlers. *Am Fam Physician* 2002 Oct 1; 66(7):1217-1424.
27. **Siti-Noor AS, Wan-Maziah WM, Narazah MY, *et al.*** Prevalence and risk factors for iron deficiency in Kelantanese pre-school children. *Singapore Med J* 2006 Nov; 47(11): 935-9359.
28. **Kilbride J, Baker TG, Parapia LA, *et al.*** Anaemia during pregnancy as a risk factor for iron-deficiency anaemia in infancy: a case-control study in Jordan. *Int J Epidemiol* 1999; 28(3):461-468.

29. **Lozoff B, Kaciroti N, Walter T.** Iron deficiency in infancy: applying a physiologic framework for prediction. *Am J Clin Nutr* 2006 Dec;84(6):1412-1421.
30. **Keskin Y, Moschonis G, Dimitriou M, *et al.*** Prevalence of iron deficiency among schoolchildren of different socio-economic status in urban Turkey. *Eur J Clin Nutr* 2005 Jan; 59(1):64-71.
31. **Kemmer TM, Bovill ME, Kongsomboon W, *et al.*** Iron deficiency is unacceptably high in refugee children from Burma. *J Nutr* 2003 Dec; 133(12):4143-4149.
32. **Fucharoen S, Winichagoon P, Wisedpanichkij R, *et al.*** Prenatal and postnatal diagnoses of thalassemias and hemoglobinopathies by HPLC. *Clin Chem* 1998 Apr; 44(4):740-748.
33. **Samavat A, Modell B.** Iranian national thalassaemia screening programme. *BMJ* 2004 Nov 13; 329(7475):1134-1137.
34. **Ho PJ, Hall GW, Watt S, *et al.*** Unusually severe heterozygous beta-thalassemia: evidence for an interacting gene affecting globin translation. *Blood* 1998 Nov 1; 92(9):3428-3435.
35. **Ahmed S, Saleem M, Modell B, *et al.*** Screening extended families for genetic hemoglobin disorders in Pakistan. *N Engl J Med* 2002; 347(15):1162-1168.
36. **Rogers M, Phelan L, Bain B**. Screening criteria for beta thalassaemia trait in pregnant women. *J Clin Pathol* 1995 Nov; 48(11):1054-1056.
37. **Telmissani OA, Khalil S, Roberts GT.** Mean density of hemoglobin per liter of blood: a new hematological parameter with an inherent discriminant function. *Lab Hematol* 1999; 5:149-152.
38. **Maeda M, Fukunaga Y, Kaize K, *et al.*** Two children with thalassemia identified during screening for anemia in junior high school. *J Nippon Med Sch* 2004 Aug; 71(4):297-300.