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SERUM IRON AND IRON BINDING CAPACITY IN MALAYANS.

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Studies on serum iron levels have been carried out in Malaya by Tasker (1955) in non anaemic patients and in nutritional anaemias. Serum iron has not been estimated in other types of anaemia and all studies carried out by Tasker applied to adults only. No work has been carried out in children and iron binding capacity has not been estimated in any condition.

The study described in this paper was done with the following purpose: (a) to establish not only the serum iron but also the serum iron binding capacity in normal healthy Malayans, (b) to study the serum iron and iron binding capacity in anaemias in children in order to evaluate the incidence of iron deficiency (c) to study the serum iron and iron binding capacity in Malayan new borns in order to see how in general, babies in this country start their life as regards the amount of iron in their blood. (d) to study the iron and iron binding capacity in other conditions, in order to check whether the results we obtained agree with those already reported by others.

METHODS AND MATERIALS

Methods. Since iron is such an ubiquitous element and its amount in serum so small, it became absolutely necessary that, in order to prevent extraneous contamination during the determination of serum iron and iron binding capacity all glassware should be entirely iron free. Therefore, all syringes and glassware were washed with either hot or boiling 5 N HCL, followed by a generous rinsing with all glass distilled water. After drying, such glassware were set aside only for iron determinations. Only all glass distilled water was used to rinse glassware and to make up reagent solutions.

Serum iron estimation was carried out by the method of Ramsay (1954), in which acetate-buffered-dipyridyl solution was added to serum followed by the reducing agent sodium sulphite. Proteins were removed by heating in a boiling water bath and centrifugation. The resultant pink ferrous-dipyridl complex was measured spectro-photometrically at 520 mu and the amount of serum iron was calculated and expressed as μg/100 ml.

Unsaturated iron binding capacity was performed by the method of Ressler and Zak (1958). The procedure involved a known amount of ferrous ions being added to a buffered solution of serum in order to saturate the binding capacity of the iron binding protein (siderophilin). The excess amount of ferrous ions that did not combine with the siderophilin was then determined photometrically after reacting the mixture with dipyridl. By subtracting this observed amount from the known amount of ferrous ions put in at the beginning of the experiment, the amount that had combined with the siderophilin may calculated. This constitutes the unsaturated iron-binding capacity and was expressed as μg/100 ml. (μg per cent). The total iron binding capacity is the sum of serum iron and unsaturated iron binding capacity.

Material. All blood samples were taken in the morning at about 10 a.m. to minimize the influence of diurnal variation (Heilmeyer and Plötner 1937, Hemmeler 1944, Höyer 1944, Waldenström 1946, Hamilton et al 1950, Paterson et al. 1952, Howard 1953, Antila 1962).

The material for study consisted of four different groups, a group of normal healthy individuals, a group of anaemic children, a group of newborns and their mothers, and a group of miscellaneous diseases.

The normal healthy non anaemic person were obtained from the blood bank. Only blood donors who came for the first time were considered for study. This group also consisted of laboratory workers and administrative personnel. They were all adults, ranging in age between 17 and 50 years.

The group of anaemic children were obtained from the paediatric wards of the General Hospital, Kuala Lumpur. They were of the middle and lower income groups and they ranged in age between 3 months to 7 years. These children were taken at random the only criterion being that the haemoglobin level was below 8 g %.

Newborns and their mothers were obtained from the labour room in the General Hospital. The mothers were in parturition and the blood was taken only in the morning from them. Blood specimens of the newborns were taken from the cord and were divided into two groups, those born in the morning and those born in the afternoon or evening.

RESULTS

A total of 324 persons have been examined for serum iron and serum iron binding capacity. The results in 136 normal healthy persons, 131 males and 5 females, are presented in table 1. The males are divided into six racial groups, 48 Chinese, 39 Malays, 29 Indians, 4 Sikhs, 6 Eurasians, and 5 Europeans. The number of females examined was too small for consideration. They are put into one group.

A total of 97 anaemic children were examined. Four of them were excluded because it was found that they were under iron treatment when the blood was obtained. The results in the remaining 93 are found in table 2. They are grouped according to haematological diagnosis. A group of miscellaneous diseases in these children consists of a number of diseases (too few of each for proper evaluation.)

The cord bloods of 32 newborns and peripheral blood of 36 mothers were studied. 26 of the mothers were examined before delivery, 10 after delivery. In 6 mothers the babies blood was not examined. Of 2 babies the mother was not examined. 24 of the babies were born in the morning, only 2 were born in the afternoon. In 6 the time of delivery was not recorded. The results are presented in table 3.

The findings in 23 miscellaneous diseases obtained from different wards of the General Hospital are not presented in a table because the number of each disease is considered too small to be of much importance.

DISCUSSION

Normal persons. In the three representative ethnic groups in Malaya, the Chinese, Malays, and Indians, the mean for serum iron was found to be respectively 123 ug per cent, 112 μg per cent and 114 μg per cent. These are within the normal range when compared with those reported by many other workers from different countries. In Europeans, the mean for serum iron in men reported in 37 papers from 8 different countries (compiled by Viljo Antilo 1962), ranged between 100 and 146 μg per cent. In Asia, Agarwal and Misra (1955) found a mean of 117 μg per cent for 50 men in India, and Sakakura found a mean of serum iron of 121 μg per cent in 20 male Japanese. In Malaya, Tasker (1955) examined patients with haemoglobin levels of higher than 14 g % and found a mean serum iron of 145 μg per cent. They were in fact not normal and healthy individuals, since they came to the hospital for one or other complaint.

Of the three representative groups in our series, the Chinese had the highest mean serum iron level, the Malays and Indians had about the same mean serum iron. There is some indication that the Europeans had the highest mean serum iron, while the Sikhs had the lowest of the whole series examined. However, the numbers of persons examined in these last mentioned groups were too small to warrant any conclusion.

As regards the total iron binding capacity, the values obtained by us, 318 μg per cent in Chinese, 325 μg per cent in Malays and 326 μg per cent in Indians are again within the normal range when compared with those reported in 12 papers from European countries, which ranged between 253 μg per cent and 348 μg per cent (Antila (1962).

Anaemic children. A total of 97 anaemic children with haemoglobin below 8 g % were examined. Four were excluded because they were on iron treatment when the blood was taken. The mean serum iron in the rest, a total of 93 children, was 76 μg per cent with a range of 10 μg per cent to 363 μg per cent.

TABLE I
Serum Iron and Total Iron Binding Capacity (TIBC)
in Normal Healthy Malayans

Race	Sex	No. exam.	Serum Iron µg%			TIBC µg%		
			Mean	Range	SD	Mean	Range	SD
Chinese	M	48	123	45-240	49.1	318	217-508	78.6
Malay	M	39	112	67-228	37.1	325	218-392	68.8
Indian	M	29	114	60-180	32.4	326	270-441	59.5
Sikh	M	4	89	40-120	37.3	410	293-502	105.1
Eurasian	M	6	128	94-202	45.7	341	258-484	88.9
European	M	5	132	64-177	46.3	299	233-344	45.4
Total	M	131	117	40-240	41.6	324	217-508	62.3
Total	F	5	105	75-160	32.4	334	284-368	36.4

TABLE II
Serum Iron and Total Iron Binding Capacity (TIBC)
in Anaemia Children

Haematological Classification	No. and Race	Serum iron µg%			T.IBC µg%		
		Mean	Range	S.D.	Mean	Range	S.D.
Iron def. anaemia	49 (M9 C10 I30)	40	10-131	24.0	364	250-509	74.2
Megalobl. anaemia	15 (M3 C1 I10 P1)	62	20-154	39.4	367	184-439	76.1
Haemolytic anaemia	9 (M2 C6 A1)	174	56-343	101.4	266	164-351	68.1
Malaria	6 (M4 C1 I1)	145	40-363	92.1	318	243-512	100.4
Leukaemia	3 (all C)	107	60-144	43.0	372	336-360	43.8
Miscellaneous	11 (M3 C7 I1)	125	31-471	129.5	298	144-516	133.5
Total	93	76	10-363	77.7	345	106-516	88.8

M = Malay
C = Chinese
I = Indian
P = Pakistani

TABLE III

Serum iron and Total Iron Binding Capacity (TIBC) in Newborns and their Mothers

	time blood taken	No. and Race	Serum iron μg%			TIBC μg%		
			Mean	Range	S.D.	Mean	Range	S.D.
Newborns	at birth in morning	24 (M1 C16 15 E2)	171	40-308	67.3	306	121-483	82.5
	at birth in afternoon and night	2 (I2)	165		—	274		45.3
	Unknown	6 (C3 13)	106	60-200	58.5	336	204-471	109.7
	Total	32 (M1 C19 I10 E)	158	40-308	67.6	310	121-483	85.5
Mothers	Before delivery in the morning	26 (M2 C18 I6)	96	28-210	53.2	452	339-566	58.2
	After delivery in the morning	10 (C7 I3)	64	20-140	38.6	368	149-495	101.2
	Total	36 (M2 C25 I9) 36	87	20-210	51.2	431	149-566	79.2

M = Malays
C = Chinese
I = Indians
E = European

49 of them had serum iron levels below 50 μg per cent. That is, more than half had very low serum iron levels. In the group classified as iron deficiency anaemia from haematological studies, the mean serum iron was 40 μg per cent and the mean total iron binding capacity 364 μg per cent. That the mean serum iron of the whole series is not as low as expected with such a large proportion of the children having a low serum iron level is due to the increase of serum iron in 15 cases of haemolytic anaemia, 9 congenital and 6 due to malaria. If these haemolytic cases were taken apart, the mean serum iron of the rest would have been 62 μg per cent and the total iron binding capacity 356 μg per cent, giving a low mean saturation of 17%. These fiindings clearly demonstrate the importance of iron deficiency in anaemias in children in this country. It could be argued, that many of the children admitted to the hospital must have had one or other infection accounting for a low serum iron level. However, it can be seen from the unsaturated iron binding capacity, that most of them had a high unsaturated iron binding capacity, except the group of haemolytic anaemias. Only 2 of those with low serum iron level had an unsaturated iron binding capacity below 200 μg per cent, while the mean unsaturated iron binding capacity from the whole non haemolytic group is 294 μg per cent. This indicates that the low serum iron in the majority in this group was indeed due to iron deficiency, since in infection, a low unsaturated iron binding capacity is usually found. We have also been able to further check it by clinical and haematological means, for instance by studying the haematological picture and response to iron treatment. From studies of the faeces for hookworms eggs in these children (Lie-Injo Luan Eng and Virik, Lie Kian Joe 1963) it has to be concluded that hookworm is of no importance in the causation of anaemia in children below 2 years in Kuala Lumpur and environment. In children between 2 years and 7 years, there may be some influence, but in general, it was considered not to be great. The major cause for iron deficiency must therefore be sought in nutrition. Whether this is due to actual deficiency of iron in the daily diet of the Malayan child or to the factor of poor absorption due to the presence of substances taken at the same time in their daily food, which precipitate the iron present in the diet, such as phytic acid, can not be said from this study. However, Thomson (1960) and Sedky (1962) found that the iron in the daily diet in Malayans in general, is below standard requirements.

The four cases who were found to be on iron treatment when the blood sample was taken, were excluded from the list in order not to obscure the findings. Two of these cases were found to have extremely high serum iron levels of 600 μg per cent and 450 μg per cent.

In anaemia with megaloblastic changes of the bone marrow, the mean serum iron was 62 μg per cent in 15 children examined. It must be mentioned however, that the megaloblastic changes were mostly of the intermediate type and that they were in addition to an iron deficiency state in 12 of the cases. The unsaturated binding capacity was also high in this series, indicating an iron deficiency state.

In haemolytic anaemias most of them congenital (6 thalassaemia major, 2 Hb E-thalassaemia and 1 auto-immune haemolytic anaemia) the serum iron was definitely increased with a low unsaturated iron binding capacity. The same can be said of the cases of malaria which is essentially haemolytic in nature. As can be seen from the range of serum iron values, not all of them showed a high serum iron level. This probably depended upon the haemolytic activity at the time the blood was obtained.

Newborns and their mothers. The mean serum iron in newborns was 158 μg% (see table 3) that is, higher than the mean in normal male adults. This is in agreement with those reported by others (Hagbergh 1953, Sturgeon 1954). It is also higher than the mean serum iron in the mothers in whom a mean serum iron level of 87 μg per cent was found. A proportion of the babies we examined, had a very low serum iron level at birth. No comparison can be made between the serum iron levels of babies born in the morning with those born in the afternoon since only two samples were from babies known to be born in the afternoon. The mean total iron binding capacity in our newborn babies was normal. In the mothers the mean serum iron was found to be lower than in normal males

Blood samples taken before delivery gave a higher mean serum level than in those taken after delivery. The total iron binding capacity was relatively high. The findings in this group are difficult to evaluate, since the factor of iron treatment is unreliable. Most of the mothers visited antenatal clinics and were given ferrous iron tablets prior to delivery, but very often this was not recorded. The mothers, when asked whether they had had iron treatment, usually did not know, since they did not know whether the tablets they received from the clinics or from their private doctors or which they bought from the dispensary at the advice of friends, did contain iron. It was because of these difficulties that we stopped studying this group further. The mode of transmission of iron from mother to her foetus is still obscure. Our number of estimations is too small to warrant conclusions. However, it would be interesting to study this problem further, in more detail and on the basis of more reliable data as regards iron treatment prior to delivery.

Miscellaneous diseases. A total of 23 patients with different diseases were examined. In general the results of our study in this group agreed with those reported by others. For instance in three cases of infectious hepatitis, two were found to have much increased serum iron level and the mean in this group was 176 ug per cent. In 5 cases of leukaemia the serum iron level was found to be within normal limits and the mean was 104 ug per cent. The mean total iron binding capacity was also normal. In two cases of autoimmune haemolytic anaemia and 2 cases of haemoglobinopathy in adults, the serum iron was much increased. The same can be said of cases of malaria in adults. The unsaturated iron binding capacity in these haemolytic conditions accordingly was relatively low. We did not find an increase of serum iron in 9 cases of thalassaemia trait carriers, but the number examined is too small included 3 females, 2 examined soon after delivery. The number of each disease examined in this miscellaneous group was too small to enable a proper evaluation of the findings.

Conclusion. Several useful data were obtained from this study. The normal values for serum iron and iron binding capacity in Malayan males were established and were found to be similar to those reported by other workers. The importance of iron deficiency in anaemias in children was clearly demonstrated and an idea as regards the serum iron and iron binding capacity at the start of life in new borns was obtained.

SUMMARY

A study of serum iron and iron binding capacity was carried out (a) in normal healthy Malayans, (b) in anaemic children admitted to the General Hospital, (c) in newborns and their mothers and (d) in a group of miscellaneous diseases.

The mean serum iron in the normal healthy males was as follows: 123 μg per cent in 48; Chinese, 112 μg per cent in 39 Malays, 114 μg per cent in 29 Indians 89 μg per cent in 4; Sikhs 128 μg per cent in Eurasians and 132, μg per cent in 5 Europeans. The total iron binding capacity in the same groups were as follows: 318 μg per cent in Chinese, 325 μg per cent in Malays, 326 μg per cent in Indians 410 μg per cent in Sikhs, 341 μg per cent in 6 Eurasians and 299 μg per cent in Europeans.

The findings in anaemic children clearly demonstrate the importance of iron deficiency in the causation of anaemia in children in Kuala Lumpur and environment, which was most probably due to a deficient diet. The mean serum iron in this group is 76 μg per cent with a range of 10 μg per cent to 363 μg per cent, 49 of these falling below 50 μg per cent. According to haematological classification 49 had iron deficiency anaemia, without megaloblasts in the bone marrow, 15 had megaloblastic changes of the bone marrow, 12 of whom in association with iron deficiency.

In congenital haemolytic anaemia and malaria in these children, the serum iron was high with a low unsaturated iron binding capacity.

In 32 newborns the serum iron was found to be generally high, higher than those of the mothers. A number of the newborns had a relatively very low serum iron level.

A group of 23 miscellaneous diseases gave results in general in agreement with those reported by others but the number of cases examined of each disease was too small to warrant conclusions.

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