Complement system in iron deficiency anemia

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Summary. Serum complement (CH50) and C_3 were assayed in iron deficient anemic subjects. The results showed that their levels were low compared to normal controls. Adding iron in vitro to serum and activating the complement components, in order to explain the above observation, had no effect.

Anemia is a major public health problem in developing countries[2] and iron deficiency appears to be the most important cause. Several reports show that host defence mechanisms are altered in iron deficiency anemia. Bactericidal activity of leukocytes and the cell mediated immune response have been shown to be impaired[3]. Serum complement levels were also found to be reduced in anemic patients[4]. However, most of the data was obtained from subjects with severe anemia. The present study was undertaken in children with varying grades of anemia to detect the level of hemoglobin at which the complement system is affected. In vitro studies were also performed to investigate the mechanisms involved.

Material and methods. A total of 98 children aged between 3 and 10 years were investigated. All of them belonged to a low income group; their weights were above 80% of Indian standards. Those who had overt infection were excluded from the study. Blood samples were collected from all the subjects in order to estimate hemoglobin and serum iron concentration. Total hemolytic activity was determined by the method of Mayer[5], and C_3 levels were determined by radial immunodiffusion[6]. In 10 subjects who had Hb-values below 10 g/dl and a serum iron concentration below 80 µg/dl, further studies were done to determine the effect of in vitro addition of iron on serum complement activity. The serum samples were subjected to adsorption with sheep RBC to remove natural hemolysins and then incubated with varying levels of added iron in the form of ferric chloride. The levels ranged from 5 to 500 times the serum iron concentration. The experiments were conducted at 2 different temperatures; one set of samples was incubated at 4 °C overnight and the other set at 37 °C for 1 h. After incubation, the complement titration was carried out as usual. A control tube without any additional iron was run with each experiment.

Total hemolytic complement (CH50) and C_3 levels in serum of children with different hemoglobin (Hb) levels

Group	Hb (g/dl)	CH50 (units/ml)	C_3 (mg/dl)
A	> 12	64.3 ± 2.49 (28)	1.2 ± 0.15 (8)
B	10-11.99	51.2 ± 2.59 (17)	1.1 ± 0.29 (3)
C	8- 9.99	50.5 ± 3.41 (15)	0.95 ± 0.13 (11)
D	6- 7.99	49.1 ± 4.48 (18)	0.79 ± 0.03 (7)
E	< 6	53.3 ± 3.76 (20)	0.68 ± 0.06 (7)

Values are mean ± SE (numbers in parenthesis indicate the sample size)

Statistical analysis by t-test:

Group	CH50	C_3
A vs B	$p < 0.001$	NS
A vs C	$p < 0.001$	NS
A vs D	$p < 0.001$	$p < 0.02$
A vs E	$p < 0.001$	$p < 0.05$

Results. Children with Hb-levels of more than 12 g/dl were considered as normal controls. Others were divided into 4 groups with a difference of 2 g Hb (table). Serum iron concentration was less than 100 µg/dl in all the anemic patients, confirming iron deficiency. The mean levels of CH50 were significantly lower in all 4 groups of the anemic subjects compared to the control group. C_3 concentration showed a progressive decrease with decreasing Hb-levels, and the reduction was significant in those who had levels below 8 g/dl.

The results of in vitro studies showed that there was no change in serum complement activity after addition of iron. The serum tended to precipitate at a higher concentration of iron.

Discussion. These data indicate that serum complement activity is reduced in nutritional anemia, confirming the earlier observations[4]. Alteration in CH50 was noted in all groups with Hb less than 12 g/dl, indicating that the complement system is affected even in milder grades of anemia. These results also suggest that a reduction in complement activity may be due to a decrease in C_3 concentration. The exact role of iron in complement activation is, however, not clearly understood. It is known that the first step in activating the complement cascade involves 2 divalent cations - calcium and magnesium. It was considered possible that iron may also have similar role. Since complement components are known to be unstable at room temperature, in vitro incubation was carried out at 4 °C. There was no change in the complement activity after addition of iron. Other workers have carried out such studies at 37 °C, reducing the incubation period[7]. When we repeated the experiment by incubating the samples at 37 °C for 1 h the results were not different. These observations indicate that iron may not have a direct role in the activation of complement.

Complement levels are restored to normal after iron therapy[4]. Administration of medicinal iron is known to stimulate protein synthesis[8]. An increase in synthesis of complement proteins may be one of the possible factors responsible for improving complement activity. Further studies were needed to understand the mechanisms involved.

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2 WHO Tech. Rep. Ser. No. 405 (1968).
3 Srikantia, S.G., Siva Prasad, J., Bhaskaram, C., and Krishnamachari, K.A.V.R., Lancet *1* (1976) 1307.
4 Jagadeesan, V., and Reddy, V., Indian J. med. Res. *70* (1979) 745.
5 Mayer, M.M., in: Experimental immunochemistry, Chapter 4, p. 133. Eds E.A. Kabat and M.M. Mayer. Charles C. Thomas, Springfield, Ill. 1961.
6 Mancini, G., Carbonara, A.O., and Heremans, J.F., Int. J. Immunochem. *2* (1965) 235.
7 Lew, F.T., Yukiyama, Y., Waks, H.S., and Osler, A.G., J. Immun. *115* (1975) 884.
8 Weisenberg, E., Halbreich, A., and Magor, J., Biochem. J. *188* (1980) 633.

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