# SERUM HAPTOGLOBIN LEVELS AND TYPES IN NORMAL MALAYSIANS

By
J. GANESAN
M.B.B.S., D.C.P., D.Path.,
WONG PUI WAN
and
LIE-INJO LUAN ENG
M.D., Ph. D. D.T.M. & H.

Divisions of Haematology and Blood Genetics, Institute for Medical Research, Kuala Lumpur.

This work was supported by the University of California International Center for Medical Research (ICMR) with Research Grant A1-10051 from the National Institutes of Health, US Public Health Service.

Polonovski and Jayle (1938)[1] first detected the presence in the serum of a protein having the property of binding haemoglobin. Subsequently these authors[2] characterized the binding substances as an $\alpha_2$ glycoprotein and gave it the name of haptoglobin (HP). The ability of haptoglobin to bind haemoglobin serves to prevent haemoglobin released into the plasma from being excreted by the kidney, through the formation of a larger molecule, the haemoglobin-haptoglobin complex. [3,4] This haemoglobin-haptoglobin complex is cleared from the plasma much more rapidly than free haptoglobin, so that marked hypohaptoglobinaemia is a concomittant of haemolysis. [3,4] If all the available haptoglobin has been consumed in forming this complex, as in cases of severe haemolysis, then the unbound haemoglobin in the plasma is free to pass into the urine, and haemoglobinuria will be observed. This state exists until the liver is able to produce sufficient haptoglobin to combine with and surpass the haemoglobin present in the plasma. This process takes about 38 hours but normal levels are not reached until about 4 – 7 days after a haemolytic episode.[5]

In 1955 Smithes[6] showed on starch gel electrophoresis that there were 3 main types of haptoglobin. He designated them Hp 1 – 1, Hp 2 – 1 and Hp 2 – 2; and subsequently several different sub-types have been demonstrated.

A number of clinical conditions have been shown to cause a rise or fall in the serum haptoglobin level. But the normal serum haptoglobin level in Malaysians has not so far been determined. We therefore set out to determine the normal range of serum haptoglobin in the three different racial groups in Malaysia. At the same time we determined the haptoglobin types in the same individuals so as to see whether the serum haptoglobin level is related to the genetic haptoglobin type, as has been reported previously.[7,8,9]

## MATERIALS AND METHODS

The serum haptoglobin level was determined in 240 male Malaysian blood donors of whom 79 were Malays, 81 Chinese and 80 Indians. Their ages ranged between 18 and 57. They had a haemoglobin level of over 13 gm %. A haemoglobin analysis was done on all these donors and this included alkalic denaturation for haemoglobin F, haemoglobin $A_2$ quantitation and electrophoresis of haemoglobin on starch gel. Persons found to have an abnormal haemoglobin were excluded from the series. We also excluded those found to have increased levels of Hb

$A_2$ or F; thus persons with — thalassaemia trait were as far as possible excluded. Motulsky's test for G6PD deficiency was also done on all these donors and those with G6PD deficiency were excluded.

The serum haptoglobin level was measured by the method of Owen *et al.*[10] This is based on the fact that the complex formed by the combination of serum haptoglobin with methaemoglobin has a much greater peroxidative activity than free methaemoglobin; the peroxidative activity of the complex is measured with a spectrophotometer and the concentration of haptoglobin determined by reference to a calibration curve. The values are expressed in terms of bound methaemoglobin as mg per 100 ml of serum.

The haptoglobin types were determined by starch-gel electrophoresis by the method of Poulik.[11]. A highly sensitive benzidine solution was used to stain the haemoglobin-haptoglobin complex.

## RESULTS

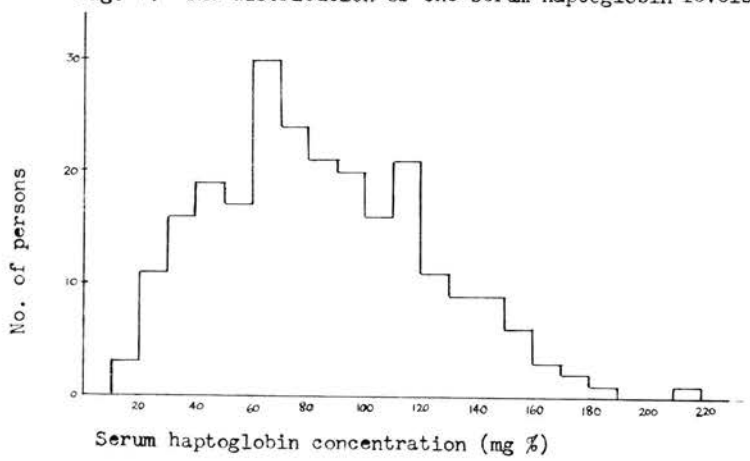
Table 1
Serum haptoglobin levels in normal Malaysians

| Race | No. of subjects examined | Serum haptoglobin concentration (mg/100ml) | | |
|---|---|---|---|---|
| | | Mean | S.D. | Actual range |
| Malays | 79 | 89.0 | 37.1 | 18.0 – 212.5 |
| Chinese | 81 | 84.8 | 38.7 | 17.2 – 176.6 |
| Indians | 80 | 82.0 | 37.3 | 21.7 – 169.7 |
| All three races | 240 | 85.3 | 37.0 | 17.2 – 212.5 |

Table 2
The relationship of the serum haptoglobin level to the haptoglobin type.

| No. examined. | Haptoglobin type | Serum haptoglobin concentration (mg/100ml) | | |
|---|---|---|---|---|
| | | Mean | S.D. | Actual range |
| 12 | 1–1 | 103.3 | 43.2 | 26.8 – 158.0 |
| 88 | 2–1 | 100.6 | 33.3 | 25.1 – 212.5 |
| 140 | 2–2 | 74.1 | 36.0 | 17.2 – 176.6 |

Fig. 1. The distribution of the serum haptoglobin levels.



The results of the serum haptoglobin estimation in each of the three main racial groups in Malaysia, i.e. the Malays, Chinese and Indians, and the results for the group as a whole are presented in table 1. The difference between the means for the Malays and Chinese was not significant ($p>0.25$) and neither were those between the means for Malays and Indians ($p>0.1$) or Indians and Chinese ($p>0.25$).

The frequency distribution of the serum haptoglobin levels in the whole group is shown in fig. 1.

Table 2 shows the relationship of the serum haptoglobin level to the haptoglobin type. The difference between the means for Hp 1 – 1 and 2 – 2 was significant ($p<0.05$) and that between 2 – 1 and 2 – 2 was highly significant ($p<0.001$) while that between 1–1 and 2–1 was not significant ($p>0.25$).

## DISCUSSION

It can be seen from the results that the range of serum haptoglobin in our normal population is very wide. This has in fact been so in most of the published series. Smith and Owen[7] whose method we have used had similar findings in 152 Caucasian blood donors: mean 93, S.D. 40 and actual range 4–220. Nyman[8] using the peroxidase-iodide method of Jayle[12] found again in Caucasians a mean value of 110 with a S.D. of 41 and an actual range of 20–250. In a recent report of the haptoglobin level in 200 normal Thais[13] using the method of Owen et al, the mean was found to be 90.61 with a S.D. of 26.5 and an actual range of 41.50 to 170.40. The range of values obtained in the Thais was therefore not as wide as that found by us in Malaysians or that found in the earlier mentioned series in Caucasians.

In various population surveys, it has been found that the average serum haptoglobin level as indicated by the hb-binding capacity depends on the haptoglobin phenotype: it has been found to be highest for the Hp 1–1 phenotype and lowest for the Hp 2–2; the Hp 2–1 phenotypes have intermediate levels.[7,8,9] Smith and Owen, whose method we have used, reported that the values for the group of Caucasian donors that they examined were:–

| Haptoglobin type | Mean | S.D. | Actual range |
|---|---|---|---|
| 1–1 | 104 | 34 | 21–155 |
| 2–1 | 102 | 37 | 28–220 |
| 2–2 | 72 | 38 | 14–193 |
| All | 93 | 40 | 14–220 |

Two of their sera contained so little haptoglobin (4 and 11 mg/100 ml) that it was not possible to determine the haptoglobin type.

Our findings, shown in table 2, are in agreement with the above reports that the serum haptoglobin level in terms of the haemoglobin-binding capacity depends on the genetic haptoglobin type. The lowest values for the serum haptoglobin level in our series were 17.2 and 21.7 mg/100 ml and at these levels we could still determine the haptoglobin type by starch gel electrophoresis although the pattern was faint and both these sera showed the pattern of Hp 2 – 2.

Like Smith and Owen, with the same photometric method, we also failed to find any normal serum completely lacking in haptoglobin. Several reports[14-19] have been made of Hp O in different populations. Kirk *et al.*[18] when examining serum samples from Malaysians (made up of approximately equal numbers of Malays, Chinese and Indians) by starch gel electrophoresis found a frequency of Hp 0 of 8 in 622 people. But Kirk *et al* and the other authors did not examine only healthy persons and they did not screen them for abnormal haemoglobins, – thalassaemia trait and G6PD deficiency as we have done. Also most of these authors used only starch-gel electrophoresis to determine the frequency of Hp 0. And as was pointed out by Kirk *et al.* it is difficult to determine the Hp 0 phenotype with certainty using the starch gel method as in many cases the amount of the haptoglobin present in the Hp 2 – 2 individuals is so slight that it can readily escape detection.

Because the normal range of serum haptoglobin is so wide, an isolated observation of a low haptoglobin level in any patient may not be of much value. Complete anhaptoglobinaemia however would still be significant. Also the haptoglobin level remains fairly constant in any healthy individual.[20,8] Thus observation of a marked rise or fall usually has some clinical significance. Haptoglobin measurement can be helpful in confirming that an acute haemolytic episode has taken place even when there is no longer any demonstrable free haemoglobin or methaemalbumin in the plasma, because the

haptoglobin level does not return to its usual level for 4–7 days after complete depletion unless the patient has a disease associated with increased Hp synthesis. However, as has already been pointed out above, while complete anhaptoglobinaemia is significant, interpretation of a low haptoglobin level in terms of increased haemolysis is only reliable if a specimen obtained before the haemolytic episode is available for comparison. In chronic haemolytic disease there is a continuous release of haemoglobin into the circulation, and the serum haptoglobin level is continuously depressed and the renal threshold for haemoglobin is lowered.

The serum haptoglobin reaches normal adult levels by the age of one year.[21] By far the most common cause of low serum haptoglobin is an increased Hb turnover. This may be the result of increased haemolysis of circulating red cells,[3,4] or of the increased destruction of red cell precursors before their release into the circulation, so-called ineffective erythropoiesis. As an example of the latter, in pernicious anaemia, although red cell survival time is not markedly shortened, plasma haptoglobin is consistently low, and upon specific treatment with vitamin $B_{12}$ it rises to normal levels before the shortened survival time is corrected.[22,23] A lower concentration or complete absence of serum haptoglobin has also been reported in liver cell failure;[20] such patients are usually jaundiced and it is not known whether the low values are due to impaired synthesis by the damaged liver or to increased haemolysis which frequently accompanies severe haptocellular disease.

An increased serum haptoglobin level has on the other hand been found in a variety of disorders associated with inflammation or tissue destruction and the serum haptoglobin level has been used as an index of activity of such disorders. Thus elevated levels occur in acute and chronic infection,[24–27] burns and trauma,[28] collagen disease,[29–31] scurvy, amyloidosis, biliary obstruction, renal disease, neoplasms and in lymphomas, leukaemias, Hodgkins disease and myeloma.[32,33]

It can be seen from the above that a number of conditions can cause alteration of the serum haptoglobin level. The normal serum haptoglobin is the result of a balance between the factors which tend to raise the haptoglobin level and those that lower it. Thus the serum haptoglobin is usually decreased in uncomplicated haemolysis, the decrease depending on the extent of increased haemolysis. But if the haemolytic disease is complicated by a condition giving rise to an increase of haptoglobin, the increased synthesis of haptoglobin as a response to this condition may offset the increased removal of haptoglobin from the plasma as a result of haemolysis and the haptoglobin value may then be normal, or even raised. Therefore it may be said that lowered values in the absence of hepatic disease indicate haemolysis but that normal values do not exclude haemolysis especially in the presence of systemic disorders eg. infection or malignancy.

## SUMMARY

The serum haptoglobin level was determined in 240 male Malaysian blood donors of whom 79 were Malays, 81 Chinese and 80 Indians. These donors all had a haemoglobin level of over 13 gm % and haemoglobin analysis and Motulsky's test for G6PD deficiency were done on all of them and those with abnormal haemoglobin, $\beta$ – thalassaemia trait or G6PD deficiency were excluded from the series. The serum haptoglobin level was determined by the photometric method of Owen *et al.* and the result showed : in the Malays, a mean value of 89 mg/100ml with a S.D. of 37.1 and an actual range of 18.0 – 212.5 mg/100ml; in the Chinese a mean value of 84.8 mg/100 ml. with a S.D. of 38.7 and an actual range of 17.2 – 176.6 mg/100 ml; and in the Indians a mean value of 82.0 mg/100 ml with a S.D. of 37.3 and an actual range of 21.7 – 169.7 mg/100 ml. The haptoglobin type was also determined for all these donors and it was seen that the serum haptoglobin level, as indicated by the haemoglobin binding capacity depends on the haptoglobin phenotype; it was highest for Hp 1–1, and lowest for Hp 2–2; Hp 2–1 had intermediate levels. The value of serum haptoglobin estimation in clinical practice is discussed.

## ACKNWLEDGEMENTS

We wish to thank Dr. C.G. Lopez, Director of the National Blood Transfusion Centre for providing the blood specimens.

We also wish to thank Dr. Bhagwan Singh, Director of the Institute for Medical Research for permission to publish our data.

## REFERENCES


1. Polonovski, M.and Jayle, M.F. (1938) Existance dans de plasma sanquin d'une substance activont l'action peroxydasiqui de l'he'moglobine. C.R. Soc. Biol. 129 : 457
2. Polonovski, M and Jayle, M.F. (1940) Sur la preparation d'une nowvelle fraction des protein plasmatiques, b'haptoglobine. C.R. Acad Sei. 211, 517.
3. Allison, A.C. and AP. Rees, W (1957) The binding of haemoglobin by plasma protein (haptoglobin). Brit. Med. J. 2 : 1137.
4. Laurell, C.B., and Nyman, M. (1957) Studies on the serum haptoglobin level in haemoglobinaemia and its influence on renal excretion of haemoglobin. Blood 12 : 493.
5. Noyes, W.D. and Garby, C. (1967) Rate of haptoglobin synthesis in normal man. Scand. J. Clin. Lab. Invest. 20 : 33.
6. Smithes, 0, (1955) Zone electrophoresis in starch gels : Group variations in serum proteins of normal human adults. Biochem. J. 61: 629.
7. Smith, H. and Owen, J.A. (1961) The determination of haptoglobin in normal human serum. Biochem. J. 78 : 793.
8. Nyman, M. (1959) Serum haptoglobin Methodological and clinical studies. Scand. J.Clin. Lab. Invest., 11 (Syppl. 39).
9. Planas, J., Vinas, J., Castro, S, Orribas, J.M. and Martin-Matro, M.C. (1965) The values of haptoglobin and their relation to the genetic type in a group of donors. Rev. Esp. Fcisiol. 21 : 15
10. Owen, J.A., Better F.C. and Hoban J. (1960) A simple method for the determination of serum haptoglobins. J.Clin. Path., 13, 163.
11. Poulik, M.D. (1957) Starch gel electrophoresis in a discontinuous system of buffers. Nature 180, 1477.
12. Jayle, M.F. (1951) Method de dosage de l'haptoglobine seriqui. Bull. Soc. Chim. biol., 33 : 876 – 880.
13. Areekul, S and Chantachum, Y (1972) Serum haptoglobin level in normal Thais. South East Asian J. Trop. Med. Pub. Hlth. 3:2 86–287.
14. Sutton, H.E., Neel J.V., Binson G, & Zuelzer. W.W. (1956) Serum protein differences between Africans and Caucasians. Nature 178, 1287.
15. Allison, A.C., Blumberg, B.S., & AP Rees, W. (1958) Haptoglobin types in British, Spanish Basque and Nigerian African populations. Nature 181, 824.
16. Harris, H., Robson, E.B. & Siniscalio M. (1958) Atypical segregation of haptoglobin types in man. Nature 182, 1324.
17. Kirk, R.L., Lai, L & Hogben, D.L. (1960 a) Haptoglobin groups of white Australians. Med. J. Aust. 1, 45.
18. Kirk, R.L., Lai L.Y.C., Mahmood S. and Singh R.B. (1960b) Haptoglobin types in South-East Asia. Nature 183, 185.
19. Lie-Injo L.E., Bolton J.M. and Fudenberg H.H. (1967) Haptoglobin, transfermin and serum gammaglobulin types in Malayan aborigines . Nature 215 : 777
20. Jayle, M.F. and Boussier, G. (1955) See seromucoides du sang. leur relations avec mucoproteines de la substance fondamentale du tissu conjunctiv.
21. Bergstrand, C.G.Czar B & Tarukoski P. H. (1961) Serum haptoglobin in infancy. Scand. J.Clin. Lab. Invest. 13, 576.
22. Nyman, M. (1957) Haptoglobin in pernicious anaemia. Scand.J.Clin. Lab. Invest 9 : 168.
23. Owen, J.A., Carew, J.P., Cowling, D.C., Boban, J.P., and Smith, H. (1960) Serum haptoglobins in megaloblastic anaemia. Brit.J.Haemat. 6:242.
24. Bovornkitti, S. (1962) Serum protein changes in tuberculosis with particular reference to alpha–2 globulin. Amer. Rev. Resp. Dis. 85 : 56
25. Schwantes, A, Salzano, E, Castro, I. et al (1967) Haptoglobins and Leprosy. Acta Genet. (Basel) 17 : 127.
26. Bogdanovich L (1968) Serum haptoglobin as an index of the activity of pulmonary tuberculosis. Probl Tuberk 46 : 65.
27. Murray,R.,Robinson, J. Dublin,T., et al (1966) Haptoglobin and rheumatic fever. Brit. Med. J. 3490 : 762.
28. Neuhaus, O. (1964) Biochemical significance of serum glycoproteins 3. Hepatic production of alpha-1 and alpha-2 globulins responding to injury. Proc. Soc. Exp. Biol Med. 117 : 244.
29. Turowska, B. (1969) The value of haptoglobin level determination in synovial exudates in the

diagnosis of rheumatic disease. Rheumatologlia 7 : 57.

30. Kobiela, J., Turowska B, Baniowski, A. (1966) The level of haptoglobins in serum of patients with rheumatoid arthritis. Pol.Med.J. 5:1004.

31. Boottiger, L, Malmquist, E, Olhagen, G. (1964) Serum protein-bound carbohydrates in rheumatic disease. II Evaluation of activity in rheumatoid arthritis. Ann Rheum. Dis 13:495.

32. Owen, J.A., Smith, H.Padanyl, R. and Martin, J. (1964) Serum haptoglobin in disease. Clin. Sci. 26 : 1.

33. Gurda, M. (1971) Haptoglobin level in blood serum in Leukaemia patients. Folia Hamatol., Liepzig 95, 1, 5.37 – 41.