STUDY OF SERUM COPPER LEVELS IN PATIENTS WITH PIGMENTARY DISORDERS

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Melanin pigmentation has aroused the curiosity and attention of man since ages. Leucoderma was described as white leprosy in ancient Indian Medical literature by Charaka, Sushruta and Vaghbhata. Mention of the disease was made in Vedas particularly Atharva Veda which dates back to 1400 B. C. or even earlier (Bloomfield). The pathogenesis and aetiology in most of these pigmentary disorders remains obscure.

Melanin has been shown as the principal pigment present in the skin and it has been established that melanin is formed from the amino acid tyrosine through a series of enzymatic and oxidative reactions. The pathways involved in the formation of melanin are shown in Flate No. 1. The first step in this process is (i) hydroxylation to dopa (ii) followed by dehydrogenation to the corresponding quinone called dopa quinone (iii) it is unstable and rearranges to form leuco-compound or indole compound (iv) further dehydrogenation takes place to form dopachrome (v) then followed by decarboxylation and rearrangement to 5, 6 dihydroxyindole (vi) further oxidation to, indole 5, 6 quninone. Then polymerization takes place forming the pigment.

The melanin formation is thus dependant upon (i) substrate tyrosine (ii) enzyme tyrosinase (iii) molecular oxygen. Its formation can be influenced by varying any one of the above. Since substrate and oxygen are present in adequate quantities, the malanogenesis therefore seems to depend upon the status of enzyme tyrosinase, which contains about 0.2% of copper and is synthesized by the ribosomal fractions of the melanocyte. Copper enters the melanocytes and gets incorporated with protein, forming tyrosinase. Since pigment melanin is produced by the melanocyte activity and formation of tyrosinase which depends on the amount of copper received by the melanocytes from the blood, it was felt by many workers to investigate whether pigmentary disorders bear any significant relation with the copper content in the blood serum. During the last three or four decades, a great deal of work has been carried out on copper metabolism and serum copper levels, both in normal and diseased states. In this connection some work has also been done in respect of copper metabolism in pigmentary disorders. A. M. El. Mofty (1961) from Egypt reported a tendency for law serum copper levels and high glutathione levels in cases of vitiligo. Behl. et al (1961) reported significantly low serum copper values in cases of vitiligo and beneficial results in such cases,

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with oral copper therapy. Our interest in this problem was stimulated by these reports, and we were prompted to undertake a study not only of normal subjects and patients of vitiligo, but also to include cases of higher-pigmentation as well, to review this work fully.

Before proceeding further with the plan and method of work, a recapitulation of the knowledge of copper metabolism would help in understanding this problem better. The body of an adult contains about 100–150 mg, of copper. The highest concentration is found in liver, kidney, heart, brain and pancreas. Moderate concentrations are found in the intestinal tract, lung and spleen and lower concentrations are found in muscles, skin, adrenal glands, bone and blood. The normal values for serum copper is 90–150 micrograms per cent, blood, 70–120 micrograms %, R. B. C. 71–160 micrograms % and skin copper is 0.8 mg/100 grams of dry tissue. 90% of copper in plasma is associated with the globulin fraction ceruloplasmin where as the bulk of erythrocyte copper is bound to the protein erythrocuprin. 4 purified proteins have been obtained namely, cerebrocuprin, hepatocuprein, haemocuprein and milk cuproprotein and four enzymes have been identified that are widely distributed in the body. They are ceruloplasmin (plasma) cytochrome oxidase (most cells), tyrosinase (pigmented tissue) beta-mercapto pyruvase sulfurase (kidney, liver, muscle and RBC). It is absorbed from the upper alimentary tract in the form of cupric ion, loosely bound to the albumin fraction, then converted in the liver as ceruloplasmin, within 2 hours. Ingested copper loosely bound to serum albumin is transported rapidly to the liver, bone marrow and other organs, where it is stored and incorporated into cupro-proteins. Copper is excreted through bile, urine and negligible quantities through sweat (Plate No. 2). Daily requirement is about 2 mg. of copper which amount is believed to be present even in the poorest diet. Copper is found in pulses, nuts, cereals, fish and liver. Milk is a poor source.

MATERIAL AND METHODS

Serum copper was estimated in 20 normal innividuals, in 25 cases of vitiligo and 25 cases of hyperpigmentation. The hyperpigmentation group included cases of toxic melanoderma, Riehl's melanosis, freckles, suntanning, Xeroderma pigmentosum and Berloque's dermatitis. The control group includes 17 males and 3 females between the age group 20 to 40 years. 25 cases of vitiligo consisted of 13 females and 12 males between age groups 6 to 66 years. 25 cases of hyperpigmentation included 17 males and 8 females between age groups of 15 to 62 years. Clinical diagnosis alone formed the basis of selection of all the cases. In a few cases biopsy was done to correlate with the clinical diagnosis. The technique of Gubler et al (1952) was employad for the determination of copper in the serum. In this method copper is made to liberate from proteins by incubation with hydrochloric acid and this is followed by precipitation with trichloracetic acid. Iron interferance is prevented dy addition of sodium pyrophosphate. Copper in the supernatent liquid is then determined by the addition of sodium diethly-dithiocarbamate.

The color density is read by means of Hilger's spectro-photometer. This method requires only 1 ml. of serum. During the precedure, all pecautions were taken to avoid any extraneous copper contamination. The distilled water used for the estimation was prepared by double distillation in an all-glass still.

RESULTS AND DISCUSSION

The results and their statistical analysis are shown in Tables I to 4. Estimation of serum copper in 20 normal individuals showed levels ranging from 106.3 to 147.5 micrograms% with a mean value of 123.415 micrograms%. No relationship to the age of the individual was noticed. As only 3 females were taken in the control group, no definite conclusion could be drawn as regards a variation with sex. A comparison of our normal values as assessed by other workers is reproduced below:

Name of Workers	*Serum copper level Micrograms%*
1. Chopra and Balasubramanian (1958)	80—196
2. Kumar, Gupta and Mangalik (1958)	124±25
3. Panvalkar, Kalgi & Hegiste (1961)	122±22
4. A. M. El. Mofty et. al. (1961)	157
5. Behl et. al. (1961)	106±15
6. Chitre et. al. (1963)	93—150
Our present work	106±147
	(123.415±2.82)

It is seen from the table that our normal values are in agreement with majority of other workers except those of Mofty et. al. who have reported a higher figure of 157 micrograms%. The level of serum copper in 25 cases of vitiligo varied from 95-145 micrograms% with a mean value of 155 micrograms%, of these, 13 cases showed values lower than the normal and 6 cases showed values higher than the normal. There is a tendency for low serum copper values in these patients with no statistical significance. The observations presented here are in agreement with those of A. M. El Mofty et. al. (1961) who also found no significant difference in the serum copper between vitiligenous patients and normals. These authors however found that the blood glutathione levels in vitiligo were significantly elevated. Behl et. al. (1961) however claimed to have found significantly low serum copper levels in vitiligo.

In our experiments therefore we came across some alterations in serum copper levels in vitiligo, but these were not found to be constant or statistically significant—a fact which therefore goes to point that serum copper levels are an inconstant factor, and when raised do not necessarily result in clinical improvement.

25 cases of hyperpigmentation showed serum copper values ranging between 106–160 mg.% with a mean value of 131.5 mg.%. Of these 16 cases showed values higher than the normal and 9 cases showed values lower than the normal. In this group also, there is a tendency for higher values but without any statistical significance. Lahey et. al. (1953) reported hypercupremia in a variety of pathological states including most of the acute and chronic infections, leukemia, Hodgkin's disease and in various types of anaemia.

Question therefore arises whether these statistically insignificant values could be counted upon as sufficient evidence to show the role of copper in aetiology of pigmentary disorders. Referring to beneficial results reported by Behl et. al., it is seen that complete cure was reported only in 1 out of 119 cases of low income group and the disease was stated to be only arrested in others, after treatment. It is however known that the progress in extent of this otherwise asymptomatic disease is stopped after initial spread in large majority of cases.

TABLE No. I

Serum Copper Levels of Normal Subjects

S. No.	Name	Sex/Age in years	Serum copper values Micrograms%
1.	C. S.	M/39	137.5
2.	L. K. B.	M/27	113.75
3.	V. N. S.	M/24	113.75
4.	G. M. S.	M/26	112.5
5.	H. M.	M/29	147.5
6.	A, M. D.	M/26	113.75
7.	J. S. P.	M/26	131.25
8.	V. K.	M/23	106.3
9.	B. D. S.	F/21	147,5
10.	K, N.	M/22	112.5
11.	K, M.	F/22	113.75
12.	M. I. J.	M/38	118.75
13.	B. M. S.	M/26	120.5
14.	B. V. S.	M/35	131.25
15.	R. V. C.	M/24	112.5
16.	S. P. C.	M/26	131.25
17.	B. G.	M/25	120.5
18.	P. M.	M/24	131.25
19.	S. K. C.	M/26	137.5
20.	M, D.	F/23	112.5

Maximum value is 147.5 micrograms%
Minimum value is 106.3 micrograms%
Mean value is 123.415 micrograms%
Range is 106.3 to 147.5 micrograms%

TABLE No. 2

Serum Copper Levels In Patients With Vitiligo

S. No.	Name	Sex / Age in years	Serum copper values Micrograms%
1.	K. K.	F/38	112.5
2.	B. K.	M/48	125.0
3.	S. K.	F/11	118.75
4.	V. R.	F/24	95.0
5.	V. D.	F/18	120.5
6.	K. N.	F/25	131.25
7.	B. M. S.	M/35	112.5
8.	P. W.	F/52	131.25
9.	C. K.	F/21	145.0
10.	R. P.	M/10	106.25
11.	L. D.	F/28	113.75
12.	G. D.	F/6	118.75
13.	S. D. S.	M/22	106.0
14.	V. D.	F/8	112.5
15.	C. J. K.	F/18	113.75
16.	D. K.	F/18	131.25
17.	R. C.	M/25	106.25
18.	S. D. P.	M/42	118.75
19.	T. A.	M/64	112.5
20.	C. J. K.	M/10	106.25
21.	M. L.	M/26	112.5
22.	B. S.	M/7	125.0
23.	S. D.	F/21	112.5
24.	K. R.	M/66	106.25
25.	M. K.	F/27	112.5

Maximum value is 145 micrograms%
Minimum value is 95 micrograms%
Mean value is 115.0 micrograms%
Range is 95-145 micrograms%

Similarly hypercupremia occurs in many pathological states, not having known common denominators. Its specificity in the identification or prediction of a specific condition is low and none of these cases of hypercupermia resulted in increasing the pigmentation of skin. Apparently, a number of factors, many as yet undetermined, are involved in the regulation of serum copper. In the present study hypercupremia could not be demonstrated conclusively in the group of conditions all of them having hyperpigmentation. Just as serum copper levels are influenced by a number of diverse factors, perhaps melanogenesis is also dependent upon a large number of factors unknown.

TABLE No. 3

Serum Copper Levels in Patients With Hyperpigmentation

S. No.	Name	Sex/age in years	Diagnosis	Serum copper values micrograms%
1.	K. S.	F/43	Riehl' melanosis	156.25
2.	L. R.	M/35	Berloque's dermatitis	150.0
3.	J. I. S,	M/46	Toxic melanoderma	137.5
4.	S. L,	M/62	—do—	125.0
5.	J. S.	M/26	—do—	156.5
6.	S. P.	M/22	Riehl's melanosis	137.5
7.	S. P. A.	M/23	Freckles	130.0
8.	P. M.	M/33	Berloque's dermatitis	131.25
9.	K. V.	M/29	Toxic melanoderma	160.0
10.	C. L.	M/18	—do—	125.0
11.	J. S.	F/40	Chloasma	118.75
12.	M. S.	F/30	—do—	137.5
13.	G. D. S.	M/35	Berloques dermatitis	140.0
14.	S. K.	F/19	—do—	112.5
15.	M. S. V.	M/22	—do—	118.75
16.	M. L.	M/17	Toxic melanoderma	115.0
17.	C. S.	F/30	Freckles	113.75
18.	M. K.	F/48	Riehl's melanosis	137.5
19.	M. C.	M/21	Toxicoxic melanoderma	112.5
20.	U. S.	F/18	Chloasma	106.0
21.	K. P.	M/25	Toxic melanoderma	118.75
22.	K. D.	F/17	Sun tanning	113.75
23.	J. S.	M/15	Xeroderma pigmentosum	137.5
24.	K. S.	M/24	Toxic melanoderma	125.0
25.	M. K.	M/35	—do—	140.0

Maximum value is 160.0 micrograms%
Minimum value is 106.0 micrograms%
Mean value is 131.5 micrograms%
Range is 106-160 micrograms%

TABLE No. 4

Statistical Analysis

Source	No. of observations	Range	Mean ± S. E.	Statistical test (value of P)
Normal individuals	20	106.3–147.5	123.415 ±2.82	
Patients with vitiligo	25	95–145	115.0 ±2.12	0.05
Patient with hyperpigmentation	25	106–	131.5 +3.3	0.05

The statistical analysis did not show any significant difference in the two groups from the normal.

SUMMARY AND CONCLUSIONS

Serum copper levels were determined in normal individuals, cases of vitiligo and cases of hyperpigmentation using the technique of Gubler et. al. (1952). No significant alteration from normal in serum copper levels was observed neither in case of vitiligo nor in cases of hyperpigmentation. The role of copper as a therapeutic agent in treatment of vitiligo is not supported. Further probe into this matter, may reveal some other factors responsible.

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