

QUANTITATIVE ASSAY OF FAECAL X PORPHYRIN IN NORMAL HEALTHY INDIANS, A SPECTROPHOTOMETRIC AND COMPUTERISED ANALYSIS

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The faecal X porphyrin values in 100 normal healthy Indians have been determined by urea-triton solvent extraction method and its importance in the laboratory diagnosis of variegate porphyria has been discussed. The values ranged from 0.058 to 5.424 with a mean value of 1.64 ± 1.32 microgram per gram dry weight of faeces. X porphyrin excretion was higher in males having non-vegetarian diet. It increased with age and body weight. A statistically significant co-efficient of correlation existed between all the three types of faecal porphyrins.

Key words : Porphyrias, Faecal porphyrins, X porphyrins.

During last two decades, the literature has recorded emergence of a new porphyrin excretion product that may be of clinical assistance in the diagnosis of porphyrias. This is called porphyrin-peptide conjugate (for convenience termed X porphyrin). X porphyrin was probably first detected by Eales and Sweeny¹ in the urine of a patient with variegate porphyria (VP). It is present in the liver and bile and appears in faeces largely unaltered, being remarkably resistant to the proteolytic enzymes of intestines.² It is a group of porphyrin-peptide complexes with various peptide chains or fragments of chain. Neither the precise structure nor the exact mechanism of biosynthesis of X porphyrin has been worked out, but it is believed to be a heterogenous group of porphyrin-peptide conjugates, each conjugate consisting of a peptide linked through a thio-ether bond to a vinyl group of protoporphyrin.³⁻⁵

The levels of X porphyrin are altered in a number of patients having suspected error of porphyrin metabolism especially in the cases of

variegate porphyria (VP), porphyria cutanea tarda (PCT), acute intermittent porphyria (AIP), and sometimes in erythropoietic protoporphyria (EPP).^{4,6} Clinically, clear-cut cases of variegate porphyria and porphyria cutanea tarda can be differentiated but mostly it is difficult, particularly when there are no relatives of the patient to investigate and the symptoms are vague. In such instances of clinical latency, some additional aid to the diagnosis is required. In variegate porphyria, its levels are markedly elevated.^{3,4,6,7}

Unfortunately not much literature is available on the levels of X porphyrins in normal individuals. An analysis of the limited work on this aspect depicts a marked degree of difference of X porphyrin values between different ethnic groups and populations.^{3,4} No systematic study has so far been carried out in the Indian population. It was with this intention that this study was performed.

Materials and Methods

The faecal X porphyrins were extracted and assayed according to the method of Rimington et al.³ Concomittent assay of faecal copro and proto porphyrins was also carried out utilizing Rimington's method.⁸ One hundred normal healthy subjects were selected by randomised

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sampling and subjected to this study. Persons on drugs or alcohol were excluded from the study. Statistical analysis was performed on a computer (DEC-20 system).

To determine the effect of different variables like age, weight, sex and diet on porphyrin levels, final results were compiled into these groups and analysed statistically. The mean, range, standard deviation, standard error and coefficient of variation (CV) were determined for different groups. To study the significance of difference in the values of two groups 't' test was applied and compared with standard tables. To study the quantitative relationship between different variables i.e. age and body weight on porphyrins, regression analysis was done. The method of least squares was used to fit a first degree curve to the data on the scatter diagram.

Results

The values of X porphyrin were expressed in microgram per gram dry weight of faeces. The mean X porphyrin excretion irrespective

of age, sex and dietary habits was 1.64 microgram per gm dry weight of faeces. The values ranged from 0.058 to 5.424. Table I depicts values of X porphyrin in various sex, age, weight and diet groups. The differences of values in sex and diet groups were statistically insignificant. However X porphyrin levels increased with weight and age showing statistically significant differences between groups I, II and III. There was a statistically significant co-efficient of correlation between all the 3 types of faecal porphyrins. The lines of regression were obtained as follows. X porphyrin=0.39 (coproporphyrin) +0.85; X porphyrin=0.22 (protoporphyrin) +0.52; coproporphyrin=0.29 (protoporphyrin) +0.5.

Comments

Five groups of workers have made, rather piece-meal efforts to report normal values of X porphyrins.^{3,4,6,9,10} Their studies comprised of inadequate sample size of white population. Their results have been compared with our own

Table I. Values of faecal X porphyrin concentration in microgram/gm dry weight in different groups of normal population.

Group	Number of cases	X porphyrin concentration				
		Range	Mean	SD	SE	CV(%)
Sex						
Male	59	0.058—5.424	1.728	1.287	0.022	75
Female	41	0.269—5.329	1.513	1.387	0.034	92
Age						
16-25 years	49	0.058—5.424	1.213	1.113	0.023	92
26-35 years	33	0.269—4.268	1.716	1.016	0.034	59
36 and above	18	0.316—5.329	2.662	1.778	0.099	67
Weight						
38-50 Kg	43	0.094—3.618	1.122	0.838	0.020	75
51-63 Kg	30	0.297—5.424	1.624	1.269	0.042	78
64 and above	27	0.058—5.289	2.482	1.610	6.060	65
Diet						
I Vegetarian	49	0.269—5.289	1.362	1.048	1.021	77
II Non-vegetarian	51	0.058—5.424	1.906	1.511	0.030	79

S.D.—Standard Deviation

S.E.—Standard Error

C.V.—Co-efficient of Variation

Table II. Normal values of faecal X porphyrin in microgram/gm dry weight.

Author	Number of cases	Nationality	Range	Mean	SD
Rimington et al ³ (1968)	—	—	0—8.0	—	—
Magnus et al ⁴ (1971)	11	White	0—10.0	4.072	2.86
Moore et al ⁹ (1972)	50	White	—	7.0	6.0
Elder et al ⁶ (1974)	29	White	0—20.0	—	—
Goldberg ¹⁰ (1971)	19	—	—	3.5	2.1
Present study	100	Indians	0.058—5.424	1.64	1.32

in table II. Normal values of X porphyrin in Indians is much lower. The data also indicates that the normal X porphyrin concentration occurs in a wide range. Hence, the upper limit of normal values (5.424 microgram) rather than the mean value should be kept as a normal reference baseline. Since a significant coefficient of correlation exists with other easily demonstrable faecal porphyrins, the regression equations could be used to find probable projected values of X porphyrin after quantitation of copro or protoporphyrins. This has the advantage for laboratories where more tedious X porphyrin quantitation can be avoided.

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References

1. Eales L and Sweeney GD : *S Afr J Lab Clin Med*, 1963; 9 : 261-263.
2. Belcher RV, Smith SG and Mahler R : Biliary protein bound porphyrins in porphyria variegata, *Clin Chim Acta*, 1969; 25 : 45-52.
3. Rimington C, Lockwood WH and Belcher RV : The excretion of porphyrin-peptide conjugates in porphyria variegata, *Clin Sci*, 1968; 35 : 211-247.
4. Magnus IA and Wood A : X-porphyrin and porphyrias, *Trans St John Hosp Derm Soc*, 1971; 57 : 105-114.
5. Grosser Y, Eales L and Sano S : The chemical structure of porphyrin-peptide variegata, *S Afr J Lab Clin Med*, 1971; 17 : 156-159.
6. Elder GH, Magnus IA, Handa F et al : Faecal 'X' porphyrin in the hepatic porphyrias, *Enzyme*, 1974; 17 : 29-38.
7. Eales L, Grosser Y and Sano S : The porphyrin peptides; practical implications, *S Afr J Lab Clin Med*, 1971; 17 : 160-164.
8. Rimington C : Quantitative determination of porphobilinogen and porphyrins in urine and porphyrins in faeces and erythrocytes, *Ass Clin Path Broadsheet no. 70* : 1971.
9. Moore MR, Thompson GC and Goldberg A : Amount of faecal porphyrin-peptide conjugates in the porphyrias, *Clin Sci*, 1972; 43 : 299-302.
10. Goldberg A : Porphyrins and porphyrias, in : *Recent Advances in Haematology*, Editors, Goldberg A and Brain MC : Churchill Livingstone, London, 1971; p 302-306.