Short Communication

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Biochemistry Section

Diagnostic Associations of Hypercobalaminemia in Indian Population

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ABSTRACT

Introduction: Serum cobalamin (Cbl) measurements are routinely done to rule out its deficiency. Surprisingly, a high fraction of these patients display hypercobalaminemia.

Aim: To study hypercobalaminemia in hospital referred patients and their aetiological profile.

Materials and Methods: Samples received for Cbl measurement were divided into four groups as per their Cbl levels- low <200pmol/L, normal 200-600 pmol/L, high 601-1000 pmol/L and very high>1000 pmol/L. Surplus serum was further analysed for holotranscobalamin (holo TC) and Haptocorrin (HC).

Results: High Cbl was significantly associated with chronic alcoholism, liver disease and cancers. Patients with Cbl>1000 pmol/L showed higher risk of all cancer subtypes (myeloid, lymphatic, solid tumours); with highest risk for myeloid cancers. Distinctly higher median holo TC and HC levels were observed in groups with very high/high Cbl levels. Cancer, alcoholism, liver disease, renal, autoimmune and bronchopulmonary diseases showed HC above the reference range, the highest being that of cancer group.

Conclusion: High Cbl levels are as frequent as low cobalamin levels in clinical practice, and merits a full diagnostic work up. Cancer (especially myeloid), chronic alcoholism, liver disease have showed consistent association with high Cbl and HC levels.

Keywords: Cancer, Cobalamin, Haptocorrin, Vitamin B12

INTRODUCTION

Serum Cbl (Vitamin B12) is routinely measured to diagnose or rule out its deficiency. Hypercobalaminemia (high serum vitamin B12) is considered irrelevant from clinical and diagnostic point of view. Low vitamin B12 is thought to be prevalent among Indian population due to less intake of animal source foods [1]. However, the growing number of samples for Cbl measurement have highlighted the non-dismissible frequency of patients presenting with hypercobalaminemia [2] In our laboratory, 14.3% of the patients referred for Cbl measurement have values above the reference range 201-600 pmol/L), while almost the same percentage of patients have Cbl levels below the reference range. While low circulating vitamin B12 are suggestive of anemia and neuropsychiatric disorders [3], clinical implications of hypercobalaminemia is still debatable. Vitamin B12 supplementation is rampant since it is believed to favour appetite, strength, mood, neurological function, protect against cancer and heart disease as well as antioxidant properties [4]. Increased Cbl levels and/or Cbl binding proteins have been studied in different malignancies, infectious, autoimmune, renal and hepatic diseases, but the underlying alterations in Cbl related markers have not been fully understood [5]. Independent association between hypercobalaminemia, mortality and length of stay in hospitalised patients have also been reported [6].

This study was undertaken to highlight the disease associations with hypercobalaminemia in Indian population.

MATERIAL AND METHODS

The present was a observational and descriptive study which was done for three months i.e. from August-October 2017 in which all the patient serum samples were obtained as part of routine analysis in the Department of Biochemistry, Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India after approval by the Ethics committee (letter no 828/Acad). Informed consent was taken for additional markers. Exclusion criteria were: patients < 15 years of age, incomplete medical files or on Cbl supplementation (oral/intramuscular) in last three months.

The samples were divided into four groups as per their Cbl levels: low: <200 pmol/L, normal: 200-600 pmol/L, high: 601-1000 pmol/L and very high: >1000 pmol/L. Fasting blood samples were collected in serum separator tubes, centrifuged at 3000 g and analysed for serum Cbl within six hours of sample collection on Abbott-Architect i2000SR analyser with dilution where required as per protocol. The surplus serum was removed and stored at -20°C for analysis of additional markers. All study serum samples were analysed for holo transcobalamin (holo TC), active form of vitamin B12 on Abbott-Architect i2000SR analyser. HC concentrations- a glycoprotein that carries a major part of vitamin B12,were measured by ELISA as per manufacturer's protocol [7]. Reference ranges assumed were 240-680 pmol/L and 40-150 pmol/L for holo TC and HC, respectively [3]. Relevant data was collected from the medical records. The disease diagnosis as made by the clinician was considered final without further validation.

STATISTICAL ANALYSIS

Logistic regression analysis were performed to determine the associations between high Cbl levels and the selected diagnosis, using the group with low levels as reference. Results were adjusted for age (reference age 56 years) and gender (female as reference), with corresponding 95% confidence intervals (95% CI).

RESULTS AND DISCUSSION

Amongst 500 admissions, 36.8% (n=184) were on Cbl supplementation (i.m/oral) and were excluded. The mean age of the non-supplemented population was 57.2 years and was significantly higher with higher Cbl concentrations (p<0.003). There were 44% males and 56% females in the study population.

The suggested mechanisms of hypercobalaminemia are- (a) excess vitamin B12 intake/administration (b) damage to internal reservoir (liver disease) and direct release of vitamin B12 into the plasma, (c) excess production or lack of clearance of transcobalamins (TCB), (d) functional deficit of vitamin B12- due to alterations in affinity of TCBs for vitamin B12 or quantitative deficiency of TCBs [8].

We have observed Cbl levels to be significantly associated with alcoholism, liver disease [Table/Fig-1]. Median HC levels were

above the reference range in alcoholism (700 pmol/L) and liver disease (753 pmol/L). Median holo TC was high (125-132 pmol/L), but within reference range [Table/Fig-2].

Diagnoses (N=316)	<200 pmol/L	201-600 pmol/L	601-1000 pmol/L	>1000 pmol/L
Cancer (n=61)	10	14	14	23
Age Adjusted OR (95% CI)	1.00	1.25 (0.64-2.39)	2.30 (0.66-2.58)	6.48 (2.85-12.55)
Liver disease (n=30)	3	6	9	12
Age Adjusted OR (95% CI)	1.00	1.64 (0.66-4.09)	3.65 (1.57-8.50)	8.53 (3.59-20.23)
Chronic Alcoholism (n=16)	2	3	6	5
Age Adjusted OR (95% CI)	1.00	1.35 (0.64-2.82)	3.74 (1.88-7.39)	5.74 (2.76-11.96)
Renal disease (n=25)	5	7	9	4
Age Adjusted OR (95% CI)	1.00	1.07 (0.46-2.48)	2.07 (0.95-4.56)	1.08 (0.40-2.88)
Autoimmune (n=13)	5	3	3	2
Age Adjusted OR (95% CI)	1.00	0.82 (0.37-1.89)	0.99 (0.44-2.19)	1.29 (0.54-3.15)
Lung disease (n=31)	6	9	9	7
Age Adjusted OR (95% CI)	1.00	1.15 (0.60-2.10)	1.60 (0.86-2.93)	1.90 (1.00-3.70)
Cardiovascular (n=36)	10	11	8	7
Age Adjusted OR (95% CI)	1.00	0.79 (0.45-1.45)	0.92 (0.51-1.67)	1.12 (0.59-2.15)
Diabetes mellitus (n=21)	8	6	4	3
Age Adjusted OR (95% CI)	1.00	0.59 (0.32-1.10)	0.44 (0.22-0.88)	0.63 (0.30-1.29)
Neurological (n=29)	8	6	9	6
Age Adjusted OR (95% CI)	1.00	0.67 (0.34-1.23)	1.22 (0.60-2.12)	1.39 (0.73-2.63)
Gastrointestinal (n=28)	8	7	9	4
Age Adjusted OR (95% CI)	1.00	1.01 (0.63-1.61)	1.45 (0.89-2.33)	1.11 (0.61-1.89)
Psychiatric (n=26)	7	9	7	3
Age Adjusted OR (95% CI)	1.00	1.25 (0.78-2.02)	1.37 (0.84-2.22)	0.86 (0.46-1.62)

[Table/Fig-1]: Diagnostic associations with cobalamin levels.
Crude and Adjusted OR (Odds ratio) at 95% CI (Confidence interval) were obtained by logistic regression analysis; Patients with Cbl<200 pmol/L were taken as reference group; Adjusted for age (reference 56 years, female)

Diasease categories (N=316)	HoloTc [140-150] pmol/L	HC [240-680] pmol/L
Cancer (n=61)	78 (54-140)	800 (490-1520)
Liver ds (n=30)	132 (68-320)	753 (540-1260)
Alcoholism (n=16)	125 (61-240)	700 (590-1240)
Renal ds (n=25)	120 (55-225)	770 (550-980)
Autoimmune (n=13)	110 (57-180)	700 (540-1070)
Lung ds (n=31)	93 (48-160)	705 (540-1022)
Cardiovascular (n=36)	80 (45-130)	650 (490-910)
Endocrinal (n=21)	65 (43-110)	580 (470-810)
Neurological (n=29)	83 (47-160)	580 (470-880)
GI ds (n=28)	77 (44-170)	650 (510-800)
Psychiatric (n=26)	65 (44-130)	660 (500-870)

[Table/Fig-2]: Median (interquartile ranges) levels of Cobalamin related parameters in different diseases.
Holo TC: Holotranscobalamin; HC: Haptocorrin; Ds: disease; GI: Gastrointestinal

Hypercobalaminemia was found to be significantly associated with all cancer categories- highest with myeloid, then liver metastasis, lymphoids and solid malignancies. Cbl levels were significantly associated with alcoholism, liver disease. Similar findings were explained by other studies [2,5,9]. We have demonstrated high HC levels in cancer patients. It is postulated that high HCs released by tumour granulocytes and their precursors result in elevated cobalamin levels in myeloid patients [5,10-12]. Decrease in hepatic clearance of HC-B12 complex due to poor hepatic vascularisation and/or reduction in HC receptors may result in raised Cbl levels in hepatic tumour or metastasis patients. Excess synthesis of TCBs by tumour cells or increased HCs due to induction of hyperleukocytosis, may result in high Cbl in solid malignancies [2,13,14]. HC protein originates from a variety of tissues and could be a marker for disease progression. HC levels can be further correlated with the stage of cancer and may be a candidate factor in future studies of the possible pathogenic mechanisms leading to high Cbl levels in cancer patients.

Renal disease patients in the present study showed some association, though not significant, with high Cbl levels and had HC levels in the upper reference range. This could be due to lack of clearance of TCBs from kidney damage. Hypercobalaminemia has been found to have significant association with interstitial hypertrophy with an odds ratio of 2.7 [5]. Lung (bronchopulmonary) disease also seem to be associated with higher Cbl levels , but this association attenuated when adjusted for age and gender. There may be increased HC synthesis by the inflamed bronchial mucosa and this could be the major underlying cause of hypercobalaminemia [10]. The autoimmune disease category showed association with increase serum Cbl and had elevated HC levels too. There is an increased synthesis of TCBs and HC and/or decreased clearance due to autoantibodies impairing kidney filtration and cellular uptake [13].

HoloTC levels were within range in all categories, although chronic alcoholism, liver disease , renal disease and autoimmune disease patients showed higher median holoTC values [Table/Fig-2]. Six diagnostic categories-alcoholism, liver disease, cancers (800 pmol/L), renal (770 pmol/L), autoimmune (700 pmol/L) and bronchopulmonary diseases(705pmol/L) showed median HC levels above the reference - the highest being that of cancer group. Spearman's rho correlation analysis showed positive correlations between Cbl and HoloTC (0.75, 95% CI: 0.70-.79) and HC (0.62, 95% CI (0.57-0.66).

Limitation(s)

These are hospital referred patients and do not necessarily depict the distribution of hypercobalaminemia in the population. Secondly, hypercobalaminemia is not specific for any one particular disease. Thirdly, the diagnostic categories were selected based on disease associations shown by previous studies.

CONCLUSION(S)

Present results do not advocate the use of Cbl as a biomarker for any specific disease. High serum Cbl should lead to a systematic search for a hepatic disease or a tumour, or for a hepatic localisation of a tumour. Patients with Cbl>1000 pmol/L have higher risk of all cancer subtypes (myeloid, lymphatic, solid tumours); with highest risk for myeloid cancers. HC may be a candidate factor in future studies of possible mechanisms leading to high Cbl in cancer patients.

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