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Elevated Levels of Vitamin B12 in Gastro Intestinal, Pancreatic and Hepatobiliary Malignancies

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Abstract: ***Background**: Vitamin B12 is an organic compound essential for DNA synthesis, DNA methylation and mitochondrial metabolism. Deficiency states are common. Increased levels of B12 are rare but may be seen in certain conditions like chronic liver disease, chronic kidney disease, certain inflammatory conditions, myeloproliferative diseases and solid organ cancers. **Aim**: To assess the frequency of elevated serum B12 in various GI hepatobiliary and pancreatic malignancies. **Methods and Materials**: One hundred patients with cancer and one hundred controls were assayed for serum B12. Test procedure was performed with CLIA VITROS integrated system. Subjects who were administered oral & parenteral B12 in past 3 months were excluded from study. Based on serum B12, subjects were divided into 2 groups (less than 1000 pg/ml and more than 1000 pg/ml). **Statistical Analysis**: Chi square test, one way ANOVA and software SPSS version 23 was used. Confidence interval was 95% and p value <0.05 was considered significant. **Results**: In this study, out of the 200 subjects, 100 subjects were known cases of cancers involving Gastrointestinal tract, pancreas, hepatobiliary tract and also included cancers like malignant ascites and liver metastasis (test group). The remaining 100 subjects were enrolled from master health check up that included assay of serum vitamin B12 (control group). The age and gender were matched between the test and control groups. In both the control and test groups, 58% constituted male and rest were women. Only 11% of the control group and 48% of the malignancy group had elevated serum B12 levels above 1000 pg/ml; similarly, 52% of the test group and 89% of the control group had B12 levels below 1000 pg/ml (p value < 0.0001). This statistical significance demonstrates that almost 50% of individuals with gastrointestinal cancers have higher serum B12 levels. Hepatobiliary (6%), pancreatic (11%), gastrointestinal (69%) (stomach, colon, small intestine), and other cancers were among those examined. Serum B12 levels ranged from 1023 ± 14.06 to 1154 ± 141.6 in different malignancy groups, with a peak of 2003 pg/mL in GI malignancies. **Conclusion**: Our study showed elevated serum vitamin B12 levels in solid tumors of GI tract, liver and pancreas. An elevated serum B12 level might indicate increased cancer risk and the need to screen using diagnostic methods when there is high suspicion of cancer. It is also possible to understand that people with advanced and metastatic cancer may have higher B12 levels and a worse prognosis.*

Keywords: Hypervitaminosis, Cancer, Methylcobalamin, Pernicious anemia, Neuropathy, Intrinsic factor

1. Introduction

Vitamin B12 is a water soluble organic compound and is essential as a cofactor in many cellular processes like DNA synthesis, DNA methylation and mitochondrial metabolism(1). It contains cobalt, appears red in colour and exists in several forms like hydroxycobalamin, adenosylcobalamin or methylcobalamin(2). It is helpful in myelination of nerve cells, synthesis of tetra hydrafolate and DNA synthesis. Meat, eggs and dairy products are a good source of B12 and strict vegetarians may develop deficiency of B12. The daily B12 requirement is 2-3 µg & is easily obtained from animal source. Vegans have deficient states as only a small proportion is obtained from the diet (0 - 0.25 µg). Deficiency of B12 is caused by inadequate intake, malabsorption, chemical inactivation, inherited disruption of B12 transport or intracellular metabolism (3). The common manifestation of deficiency is neuropathy and/or pernicious anemia.

Dietary vitamin B12 is protein bound and is released in the acidic environment of stomach, where it is complexed to the binding protein and transporter Haptocorrin (HC), which is also known as R binder or transcobalamin I. As the B12 R binder complex traverses the intestine, it disassociates from R - binder (HC) under the action of Pancreatic proteases and the released Cobalamin is bound by the intrinsic factor in the intestine (which is also known as S-binder). This complex is essential for the ileal absorption of Cobalamin. The digestive disorders causing vitamin B12 deficiency is labeled as food-cobalamin malabsorption (FCM). There are several disorders causing FCM (4).

After cobalamin is absorbed at the brush border membrane (BBM), it disassociates from the IF and reaches systemic circulation, where it associates with transcobalamin II (TC II). TC II is responsible for the cellular uptake of vitamin B12 in most tissues.

The transcobalamins (TCB) are 3 types: I, II, III. Types I & III are 60 -70 KDa molecular weight proteins belonging to haptocorrin super family. They are also called R-binders and found in various tissues and biological fluids. TCB I & III are derived from the granulocyte linage, which explains its increase in myeloproliferative disorders. TCB III also has a role in transport of B12.

TCB II is a 42 - 47 KDa protein which specifically transports cobalamin in serum. It is essential for the transport of vitamin B12 to cells and tissues. The production of TCB II is primarily hepatic, and secondarily from endothelial monocytes and

intestine. Several disorders are identified due to congenital deficiency of TCB II (5).

Apart from the deficiency states of vitamin B12, there are instances where serum B12 can be high. The mechanisms related to high serum cobalamin include:

a) Excess intake or administration
b) Release from internal reservoir
c) Excessive production of TCB or lack of clearance
d) Lack of TCB affinity for Vitamin B12 (6)

The usual conditions resulting in high serum cobalamin apart from excess intake could be:

- Myeloproliferative disorders, solid neoplasms, renal failure, liver disorders, inflammatory disorders and certain other neoplasms.
- In this study we estimated the levels of serum vitamin B12 in patients who have cancers involving GI, liver or pancreas compared with age & sex matched controls.

Aim: To assess the frequency of elevated serum vitamin B12 in various GI, Hepatobiliary & pancreatic malignancies.

2. Material & Methods

A total of 200 subjects were studied.

100 subjects with confirmed GI, hepatic or pancreatobiliary malignancies were analysed. Blood samples were collected after informed consent. Samples were assayed by the Biochemistry Lab, Yashoda Hospitals, Secunderabad, India. Control samples,100 in number were collected from age & sex matched subjects for statistical comparison. The control group had come for routine screening to the hospital and may be considered as healthy controls.
The age group analyzed was more than 18 years & involved both sexes.

Methodology of B12 estimation.

The principle of Vitamin B12 analysis is Enhanced Chemiluminiscence :
No special preparation was suggested for sample collection and it was done in red plain vacutainer or gold serum separator tube. Samples were centrifuged at 3500 rpm for 5 minutes to separate the serum. The VITROS Vitamin B12 test utilizes 30 µL of sample for each determination. This does not take account of the minimum fill volume of the chosen sample container. (7)

The VITROS Vitamin B12 test, including sample pretreatment, was performed using the VITROS Vitamin B12 Reagent Packs 1 and 2, the VITROS Vitamin B12/Folate Reagent Pack 3 and the VITROS Vitamin B12 Calibrators on VITROS 5600 Integrated System using Intellicheck Technology. A competitive binding immunoassay technique was used, which depends on a competition between vitamin B12 present in the sample with a horseradish peroxidase (HRP)-labelled vitamin B12 conjugate for a limited number of binding sites on a biotinylated Intrinsic Factor present in the liquid phase. Vitamin B12 present in patient samples gets released from its endogenous binding proteins by alkaline denaturation. Biotinylated Intrinsic Factor conjugate is added and incubated with the treated neutralized sample. An aliquot of the treated sample is transferred into a streptavidin coated well and B12-HRP conjugate added. Following a competitive binding reaction, the vitamin B12 Intrinsic Factor complexes are captured by streptavidin on the wells. Unbound materials are removed by washing.(8)

The bound HRP conjugate is measured by a luminescent reaction A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is indirectly proportional to the concentration of vitamin B12. (9)

Table 1: Patient Characteristics

Sno.	Total	Vit B12 <1000 pg/mL (%)	Vit B12 ≥ 1000 pg/mL (%)
N	100	52 (52)	48(48)
Gender			
Male	58	32 (55)	26 (45)
Female	42	20 (48)	22 (52)
Age			
≤50	19	14 (74)	5 (26)
51-75	68	32 (47)	36 (53)
> 75	13	6 (46)	7 (54)
Malignancy			
GI Malignancies	69	39 (57)	30 (43)
Pancreatic Malignancies	11	5 (45)	6 (54)
Hepatobiliary Malignancy	6	2 (33)	4 (67)
Others	14	6 (43)	8 (57)

Statistical Analysis:

- Sample size: Test group - 100; Control group - 100
- Software used : SPSS version 23
- Test Performed: Chi-square test, One way ANOVA
- Confidence interval is 95%, hence P value < 0.05 is considered significant

3. Results

Serum Vitamin B12 was estimated in 200 subjects; 100 of these were established cases of cancer involving gastrointestinal tract, pancreas, hepatobiliary tract and a few other cancers like malignant ascites, liver metastasis.

Hundred subjects who had undergone master health check up for routine health screening including serum B12 assay, were considered as healthy controls. The gender and age was evenly matched between the two groups. Males constituted 58% in both groups and the rest were women. Age group was predominantly similar with 68% of test group in the range of 51-75 years. (Table 1)

The estimated serum B12 levels were more than 1000 pg/ml in 48% of malignancy group and only in 11% of control group; similarly 89% of control group and 52% of test group had B12 levels less than 1000 pg/ml (p value < 0.0001). This statistical significance proves that serum B12 is elevated in

nearly 50% of patients with gastrointestinal malignancies. (Table 2). The various malignancies studied were gastrointestinal (69%) (stomach, colon, small intestine), pancreatic (11%), hepatobiliary (6%) and others. The peak serum B12 was observed in GI cancers - 2003 pg/mL and the mean elevation ranged from 1023 ± 14.06 to 1154 ± 141.6 in various malignancy groups. It was ensured that all these patients were not being supplemented with oral or parenteral B12 vitamin in the past 3 months of blood sampling. (Table 3, 4)

Table 2: Comparison Between Test Group and Control Group

Parameter	Group		P value
	Test (n=100)	Control (n=100)	
Age (years)			
≤50	19	18	0.9999
51-75	68	69	
> 75	13	13	
Gender			
Male	58	58	0.9999
Female	42	42	
Vitamin B12 (pg/mL)			
< 1000	52	89	<0.0001*
≥ 1000	48	11	

Significant difference was not found in age and gender between test and control group. Statistically significant difference was found in vitamin B12 level between test and control group

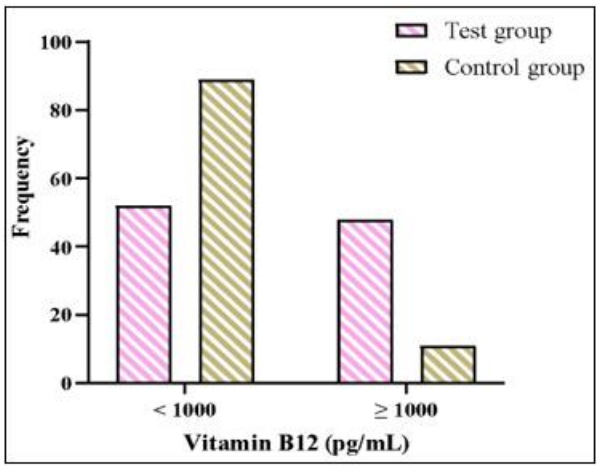

Figure 1: Comparison of Vitamin B12 Between Test Group and Control Group

Table 3: Comparison Between Elevated and Normal Vitamin B12 Subgroups in Test Group

Parameter	Vitamin B12 (pg/mL)	
	<1000 (n=52)	≥ 1000 (n=48)
Age (years)		
≤50	14(27)	05(10)
51-75	32(62)	36(75)
> 75	06(12)	07(15)
Gender		
Male	32(62)	26(54)
Female	20(38)	22(46)
Malignancy		
GI Malignancies	39(75)	30(63)
Pancreatic Malignancies	5(10)	06(13)
Hepatobiliary Malignancy	02(4)	04(8)
Others	06(12)	08(17)

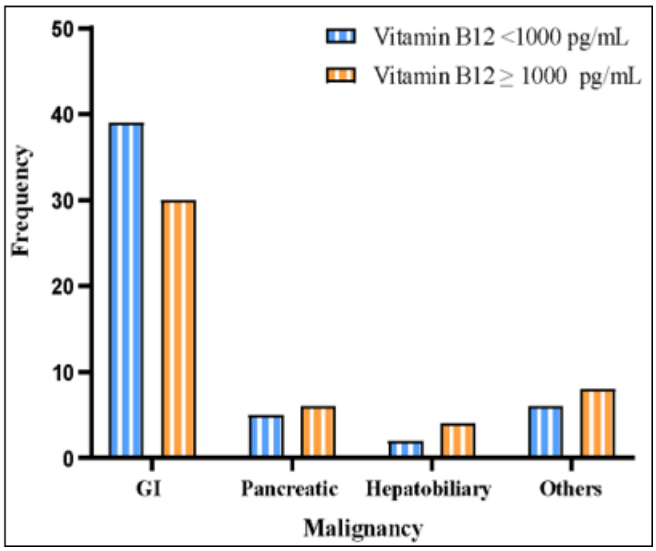

Figure 2: Comparison of Vitamin B12 in Malignancies

Table 4: Comparison of Elevated Vitamin B12 levels Between Malignancies

Malignancy	Elevated Vitamin B12 (pg/mL)			P value
	Minimum	Maximum	Mean± SD	
GI	1002	2003	1144±216.7	0.5667
Pancreatic	1021	1369	1154±141.6	
Hepatobiliary	1011	1041	1023±14.06	
Others	1012	1220	1084±82.08	

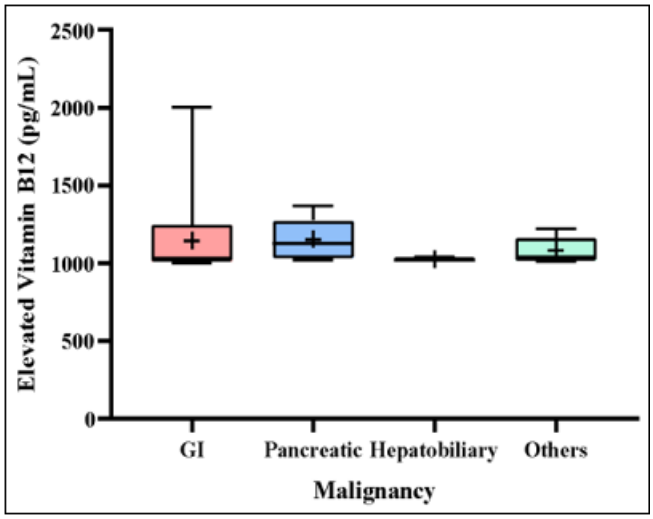

Figure 3: Elevated Vitamin B12 levels in Malignancies

4. Discussion

Vitamin B12 deficiency is important as it may cause important neurological and hematological abnormalities. As absorption and transfer of B12 is a complex process and involves many steps, deficiency state can occur when there is a defect in any of these stages. Contrary to general belief, high levels of vitamin B12 are occasionally observed and may perplex the clinician. A common cause may be excessive administration of the vitamin either orally or parenterally. But there are certain causes of elevated levels of B12 which may have prognostic implications. One such important cause is elevated level of B12 in neoplastic lesion and hematological malignancies. We focussed on finding elevated levels of B12 in Gastrointestinal, pancreatobiliary and hepatic neoplasm. More than 50% of patients with neoplasms seem to be having

a serum level of >1000 pg/ml. Finding higher B12 levels may have several implications:

a) Screening for malignant lesions (whenever suspicion is higher),
b) Presence of metastatic disease
c) Outcome (poor prognosis with higher levels)
d) Hepatic or Renal disorders
e) Excessive administration

In a study by Valentin Lacombe et al, patients were divided into three groups, normal B12 levels (n=344), persistently elevated level (n= 144) (2 elevated levels in 1 month) and non persistent elevation (n=200) (1 elevated level between 2 estimations at 1 month interval). Incidence of solid cancers was observed over 5 year period and it was noticed that solid tumor occurrence was strongly associated with the group with persistent elevation of B12.(10) In another study by Federico Sottotetti et al, 788 cancer patients were classified into 4 groups based on vitamin B12 levels as very low (14%), low (19%) normal (49%) and high (17%). It was observed that B12 levels were higher in patients with higher ECOG-PS levels and in advanced cancer compared to early cancer.(11) In a study by Geoffrey Urbanski et al, screened patients who were diagnosed to have developed solid cancers within 24 months of assay or prior to B12 assay and found a higher percentage of patients with cancer in elevated B12 group. The other causes of elevated B12 was chronic liver disease, acute liver disease, chronic kidney disease, autoimmune disease, inflammatory disease, myeloid malignancies, lymphoid malignancies and excessive supplementation of B12 either oral or parenteral. (12)

Though elevated B12 has been documented in some studies in malignant disease, there is no evidence to assume that high B12 intake or pharmacological doses is causally related to cancer. In fact a low level of B12 needs to be diagnosed in cancer patients and treated in order to prevent haematological and neurological complications.

In our study one hundred subjects with cancer and another hundred subjects who opted for general screening were checked for B12 levels. Elevated B12 was found in 48% of cancer patient group and only in 11 % of control group. A higher level of B12 seems to be commoner and found in nearly half of the patients diagnosed to have cancer. The probable cause of elevation is excessive production of transcobalamines (TCB), and a lack of affinity of TCB to B12. There is no definite evidence that tumor cells produce B12. Release from internal reservoirs like liver or kidney may be the cause in inflammatory disorders.

5. Conclusion

Elevated serum vitamin B12 levels are found in patients with malignant disease. Our study showed elevated levels in solid tumors of GI tract, liver and pancreas. When there is strong suspicion of malignancy, a finding of elevated serum B12 level should suggest intensification of cancer screening by diagnostic methods. It may also be understood that B12 levels may be elevated in advanced and metastatic cancer disease and the outcome may be poorer in patients with elevated B12 levels.

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