Original Articles

An Observational study of the Correlation of Higher Level of Serum Vitamin B 12 and Underlying Diseases

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INTRODUCTION

Vitamin B_{12} has recently gained a lot of importance in laboratory medicine owing to the magnitude of effects seen on the homeostasis and normal functioning of the various organ systems. However the focus remains on the deficiency or low serum B_{12} levels.

We discovered an unexpectedly high level of Vitamin B_{12} in the laboratory at our hospital in a group of patients and its frequent association with underlying diseases.

A follow up and detailed work up of these group of patients (after excluding the patients on therapeutic B_{12} administration) revealed a significant association with severe underlying diseases particularly hematologic and liver diseases.

A statistical analysis of this group of patients was done, and correlated with the available literature and recent studies.

AIMS & OBJECTIVES

- To study the B_{12} levels in patients during a specific time period from the laboratory records
- A detailed follow up of the patients with an unexplainable high level of B12.
- A possible disease correlation in patients with high levels
- Evaluate the significance of the disease association.

MATERIALS & METHODS

After taking an approval from the institutional ethics committee the study was conducted in two phases.

1) Analysis of the serum vitamin B_{12} levels done in the central laboratory at V.S. General Hospital.

In our laboratory, the B12 assay was carried out on 7K61 ARCHITECT B12 Reagent Kit.

2) Follow up of the patients having an unexpectedly high serum $B_{12levels}$ from the patients records.

The patients were reviewed for age, sex, dietary habits, vitamin B_{12} therapy (both oral as well as injectables), complete blood count and the associated clinical conditions or diagnosis.

RESULTS

The data of 1488 patients serum vitamin B_{12} levels as advised by the physician for a period of 3 months was analysed (July 2017 to September 2017).

The Serum B_{12} levels was categorized into 5 groups (A-E) from low to high levels.(Table 1) & the number of patients grouped into the 5 categories according to their serum vitamin B_{12} levels was listed (Table 2).

The threshold for high Serum B12 levels was taken as 1500pg/ml. (1, 2)

Hence the patients in groups D and E were followed up for the associated disease conditions.

A total of 170 patients listed in the two groups D and E were followed up, of which 19 patients belonged to group D and 151 patients belonged to group E.

The case records were reviewed for age, sex, dietary habits, vitamin B_{12} therapy (both oral as well as injectables), complete blood count and the associated disease conditions.

Of the 19 patients from group D, 10 were on injectable vitamin B_{12} therapy and were excluded from a further follow up since the high serum levels might be associated with vitamin B_{12}therapy.

Six of the patients in this group showed a functional vitamin B_{12} deficiency as reported from the peripheral blood smear picture, hematologic parameters and bone marrow examination findings. However no associated diagnosed disease condition found according to the information mentioned in the case records and follow up of the patients.

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Table 1 : Categories of patients grouped according to the Serum B_{12} levels

Group	Category	Serum Vitamin B_{12} Level (Pg)
A	Low	<200
B	Normal	200-950
C	High	951-1500
D	Very High	1501-2000
E	Extremely High	>2000

Table 2 : Number of patients in each of the groups.

Group	Serum Vitamin B12 Level (Pg)
A	642
B	636
C	40
D	19
E	151
Total Patients	1488

Table 3 : Underlying disease conditions in patients with High B_{12} levels.

Underlying Disorders	Group D (Out of 19 Patients)	Group E (Out of 151 Patients)
Vitamin B12 therapy	10	130
Functional 12 deficiency	6	12
Liver disease	2	2
Hematological diseases	1	7
Total	**19**	**151**

Table 4 : Distribution of Haematologic & Liver disorders.

Diseases		Number of cases	Total
Hematological diseases	Acute leukemias	4	8
	Chronic leukemias	2	
	HES	1	
	Atypical morphology	1	
Liver diseases	Hepatitis	3	4
	Liver abscess	1	

Functional B12 deficiency is reported when the serum vitamin B12 levels are within the normal range (on high levels). On repeated measurements along with the symptoms of megaloblastic anemia as seen from the peripheral pictures. (High MCV anemia and BM shows megaloblastic maturation. This can be confirmed by raised serum levels of MMA or homocysteine and low levels of Transcobalamine 2) (4, 5)

A failure of intracellular transport of vitamin B12 by transcobalamine-2 can lead to the same. Such patients may respond to repeated high dose injections of B12. [4,5]

Two of the patients were diagnosed cases of liver disease and 1 patient was diagnosed to have acute leukemia.

Out of 151 patients from group E, 130 patients were on injectable vitamin B12 therapy, 12 patients had functional vitamin B12 deficiency (with no associated diagnosed disease condition), 2 patients had liver disease and 7 patients had leukemia. (Table 3, Figure 1)

Out of 8 cases of hematological malignancy (group D & E), 4 patients had acute leukemias, 2 patients had chronic myeloid leukemia, 1 patient had hyper-eosinophilic syndrome (HES) and one patient had a blood picture with atypical cell morphology (the patients peripheral blood picture was suggestive of leukemia; however the patient was not available for a follow up & bone marrow examination (Table 4)

Out of 4 cases of liver diseases 3 patients had hepatitis and 1 patient had liver abscess. (Table 4)

Based on this data and as per the advice of Expert statistician (Dr. A. Tiwari Assi. Proff. Department of PSM NHLMMC) we applied the Chi square test and calculated the p value was calculated on the groups D & E (***)

By calculation we found x^2=17.263, p=0.0006 which is a significant association thereby suggests Significant association between high level of serum vitamin B12 level & the underlying diseases.

CAUSES OF HIGH SERUM VITAMIN B_{12} (1, 2, 3)

There are four mechanisms which involves the high serum vitamin B_{12} levels:

Excessive intake or therapeutic administration

Excessive liberation of vitamin B_{12} from an internal reservoir

An increase in Transcobalamin via excess production or lack of clearance

A quantitative deficiency or lack of an affinity of Transcobalamin for vitamin $B_{12.}$

Normal Range (Architect B 12 reagent Kit)

The normal values of Serum B_{12} levels range from 187 to 883 pmol/ml (138- 652 pg/L). (1, 2, 3)

The disease associations and the suggested underlying pathological mechanisms leading to elevated Cobalamine levels along with a review of the various studies are discussed.

The disease entities are represented mainly by solid malignancies, hematological malignancies and liver diseases (1, 2, 3, and 8).

Elevated B_{12} levels & Haematologic Disorders.

The suggested pathogenesis for the associations between high Cobalamine (Cbl) levels and the underlying haematological diseases involves a release of Haptocorrin (HC) from the proliferating leukocytes. (3)

Chronic myeloid leukaemia (CML) is the most thoroughly studied disease entity. Already in the 1950s, researchers showed that patients with CML had elevated Cbl levels, sometimes exceeding several thousand pmol/L.

It is hypothesised that the high levels are caused by HC release from proliferating leukocytes, although the current evidence is not as comprehensive. In addition, the diagnostic and/or prognostic values of Cbl and HC levels have yet to be recognized for these conditions (1, 2)

Several later studies confirmed that high $UB_{12}BC$ (unconjugated B_{12} Binding Capacity) and levels of Cbl support the diagnosis of CML in patients suspected for this disease. Furthermore, a measurement of these parameters could be applied to follow the course of disease (1, 2)

High Cbl and HC levels have also been described in other haematological diseases, such as polycythaemia Vera, myeloprofilerative syndrome, acute leukaemia , eosinophilia and eosinophilic leukemia.(3)

High Cbl and $UB_{12}BC$ levels have been observed in lymphoproliferative diseases, such as multiple myeloma and lymphoma. Here, the alterations were caused by either high TC levels or high HC levels. The possible sources for the high TC levels are unknown, but may relate to macrophage activity. (3, 10)

In one of the similar studies it has been documented that the patients with unexpected high Cbl levels had 4- to 18-fold higher risk of suffering from an underlying haematological disease (1, 2, and 3).

Elevated B_{12} levels and Liver diseases

Etiologically different liver diseases are associated with high Cbl levels (1, 6). The most widely studied is alcoholic liver disease. In this condition, the high plasma Cbl is associated with high HC levels, thus, possibly caused by decreased hepatic clearance or an increased release of Cbl from damaged hepatocytes.

Several studies have confirmed an association between liver cancer and the elevated levels of Cbl(1,9).The plasma Cbl level has been suggested as a prognostic marker in patients with hepatocellular carcinoma (HCC).In addition to the a release of the vitamin from damaged hepatocytes an increased HC production and/or decreased HC uptake could be involved. Interestingly, a rare form of primary liver cancer, fibrolaminar HCC, is

known to synthesize *HC*, (1, 6) and patients with this disease have shown very high levels of both Cbl, HC and $UB_{12}BC$.

Elevated B_{12} levels and Solid tumours

In addition to liver cancer, high levels of plasma Cbl has been reported sporadically in patients with lung, breast, gastrointestinal & renal cancer. However, no such case was detected in the present study. (1, 13)

Elevated B_{12} levels and autoimmune disorders

High B_{12} levels were associated with their disorders which can be studied after a follow of the various disease conditions

In autoimmune disorders both production of TC and HC may lead to high Cbl levels.

A third mechanism may also be involved – decreased TC clearance due to auto-antibodies impairing renal filtration and possibly cellular uptake (1).

Elevated B_{12} levels and renal diseases

In the early 1960s, Matthews and Beckett found elevated Cbl levels in diabetic patients with renal disease and later expanded their studies to show high plasma Cbl also in other patients with renal diseases(1).

They suggested their findings to be caused by a decreased renal Cbl clearance.

TC has a molecular mass of 38 kDa and is filtered in the kidney. This in turn may explain the high Cbl levels in patients with an impaired kidney function. The apparent size of the highly glycosylated HC is much larger (>70 kDa), and hence not filtered in the kidney (1, 10).

Elevated B_{12} levels and Infectious Diseases

The associations between infectious diseases and elevated plasma Cbl are probably multifactorial and the evidence of any underlying pathogenesis is sparse.

Both malarial infection and typhus has been related to high Cbl and TC levels (1).

In another study it was suggested that elevated vitamin B12 serum levels are associated with systemic inflammation and mortality. Since venous thromboembolism (VTE) is associated with systemic inflammation and mortality as well, they hypothesized that it is also associated with elevated vitamin B12 serum levels in elderly patients following major orthopedic surgery of the lower limb. They concluded that symptomatic VTE is associated with elevated vitamin B12 serum levels in elderly patients following major orthopedic surgery of the lower limb(7).

Elevated B_{12} levels and Liver Disorders.

The use of Cbl levels as a prognostic marker of mortality has been explored in different patient groups.

In five independent cohorts of cancer patients, high Cbl levels were positively associated with mortality risk, mainly in patients with HCC or with hepatic metastases. These observations led to the introduction of a new index, the Cbl levels times the C-reactive protein levels. This index has shown to be of some value as a predictor of mortality, although it has not been widely introduced in the clinical setting (1, 6).

Elevated B_{12} levels and Neural Disorders.

In one of the studies it was observed that vitamin B12 levels are measured when searching an origin for an anemic status (usually megaloblastic anemia), for various neurological disorders (usually polyneuropathy) or for neurocognitive disorders (8). Although the pathologies associated with vitamin B12 deficiency are well known, hypervitaminemic B12 status is often fortuitous and frequent finding.

Elevated B_{12} levels and MortalityA Study carried out on critically ill patients showed that high serum Vitamin B12 levels are associated with increased mortality in critically ill medical patients (9, 10). The author suggests that Vitamin B12 levels should be included in the work-up of all medical intensive care patients, particularly those with a chronic health history and increased severity of illness.

Elevated B_{12} levels and Lung Cancer

In one of the studies it was hypothesized to evaluate the levels of homocysteine, vitamin B_{12} and folic acid in patients with newly diagnosed lung cancer and determines whether they might be used as an accurate tumor marker for monitoring the patients if they are found to be elevated in lung cancer (11). No significant correlation was found between high B12 levels & Ca lung in their study

CONCLUSION

An unexpected high level of Vitamin B_{12} seen in patients might reflect an association with an underlying disease condition. The same has also been hypothesized as a marker of prognostic significance as well as a mortality indicator. (1, 2, 3)

Our study showed a definite association between an unexplainably high B_{12} level & associated serious underlying disorders particularly hematologic disorders, leukemia and liver disorders.

Since the B_{12} levels estimation is now carried out as a routine baseline investigations for the patients, it might be hypothesized that an unexplainably high level of the

vitamin might serve as a signal to rule out a serious underlying disorder.

Further research for the same is however required to confirm the hypothesis.

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Conflict of Interest

None

REFERENCES

1. Andrès E., Serraj K.et al. The pathophysiology of elevated vitamin B12 in clinical practice. *QJM* 2013; 106: 505–515.
2. Arendt, J. F. B. , Nexo E. Cobalamin related parameters and disease patterns in patients with increased serum cobalamin levels. *PLoS ONE 2012;* 7:e45979.
3. Arendt, J. F. B., Nexo E. Unexpected high plasma cobalamin : proposal for a diagnostic strategy. Clin. Chem. Lab. Med 2013; 51: 489–496.
4. Turner MR et al, Prac.Neurology (2009)
5. Hermann wet al.Clinchem Labmed (2003)
6. Dou, J. Et al Serum vitamin B12 levels as indicators of disease severity and mortality of patients with acute-on-chronic liver failure. *Clin. Chim. Acta* 2012; 413:1809–1812.
7. Grossfeld, A. *et al.* Symptomatic venous thromboembolism in elderly patients following major orthopedic surgery of the lower limb is associated with elevated vitamin B12 serum levels. *Clin. Biochem.2013;* 46: 54–58.
8. Guilland, J.-C. , Aimone-Gastin I. Vitamin B12 (cobalamin). *Rev Prat* 2013; 63: 1085–1087, 1089–1090.
9. Jammal, M. *et al.* High plasmatic concentration of vitamin B12: an indicator of hepatic diseases or tumors. *Rev Med Interne* 2013; 34: 337–341.
10. Rochat, et al Therapeutic and clinical implications of elevated levels of vitamin B12. *Rev Med Suisse* 2012; 8: 2072–2074, 2076–2077.
11. Serraj, K.et al. Hypervitaminemia B12 (high level of cobalamin): physiopathology, role and interest in clinical practice. *Presse Med* 2011; 40: 1120–1127.
12. Sviri, S. et al. Increased Vitamin B12 levels are associated with mortality in critically ill medical patients. *Clin Nutr* 2012; 31: 53–59.
13. Tastekin, D. *et al.* Plasma homocysteine, folate and vitamin B12 levels in patients with lung cancer. *Exp. Oncol.* 2015; 37:218–222.