



Assessment on Elevated Serum Vitamin B₁₂ Level in Cancer Patients at Hiwa Hospital

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Abstract

Free circulating serum Vitamin B₁₂ (Vit-B₁₂) levels is mainly used for diagnosis the deficiency of cobalamin (Cbl). However, approximately 15% of patients have hypercobalaminemia (high Vit-B₁₂ levels) without any existing consensus for its clinical implications. But elevated levels of serum Vit-B₁₂ could be a sign of severe, even life-endangering diseases such hematologic complications and numerous tumour types. In this work, we shall evaluate the diagnostic values of elevated Vit-B₁₂ levels in 50 cancer patients (solid cancer patients, non-solid cancer) with 36 healthy people as control. The data shows that the elevated Vit-B₁₂ concentration in the cancer patients was attributed, at least partly, to the cancer type or stage. Interestingly, %50 of patients had Vit-B₁₂ levels much greater than the upper limit (>489pg/ml), while the 36 control samples (apparent healthy) had normal levels 100%, within the reference intervals (141pg/ml to 489 pg/ml). We have concluded that the elevated serum Vit-B₁₂ is not related to diet intake, but most likely result of malignant processes. This work emphasizes that the possible clinical course of hypercobalaminemia should be under attention in the diagnostic process of malignancies.

Introduction

Vitamin B₁₂ (Cbl, Vit-B₁₂) is also called cobalamin as it contains the metal cobalt. It is one of the most complex vitamins, structurally and it is the largest. Vit-B₁₂ is soluble in water and it is transported to different organs and cells via bloodstream. It is an essential nutrient that plays a crucial function in maintaining healthy nerve cells and red blood cells^[1-3]. It is additionally expected to help make DNA, the hereditary material in all cells^[4].

The source of Vit-B₁₂ is exogenous mainly found in animal originated foods including meat, fish, eggs and milk or milk products. Stomach acidity produces Vit-B₁₂ from the process of digestion unique to Cbl, assisted with another proteins, R-protein haptocorrin (HC), transcobalamin I (TCB) and aka cobalophilin. After cleavage, Vit-B₁₂ combines with intrinsic factor, which is secreted by parietal cells in the gastric mucosa.

Intrinsic factor is required for absorption of Vit-B₁₂, which takes place in the terminal ileum^[5]. The transport of Vit-B₁₂ in the blood as well as its tissue and hepatic uptake requires the presence of transcobalamins (TCBs). Type I and III transcobalamins (TCB) ensure the binding of 80% of circulating Vit-B₁₂; however, transcobalamin II (TCB II) plays the predominant role in the key processes of tissue and hepatic uptake of Vit-B₁₂. While free Vit-B₁₂ is excreted in urine. The body can easily remove such excess vitamin from the blood stream and its stores large volumes of cobalamin in the liver^[6]. Large intake amounts of Vit-B₁₂ seem to be nontoxic but are not recommended. Hypocobalaminemia, reductions of Vit-B₁₂ levels are predominant in old people, human immunodeficiency virus (HIV)-infected individuals, and also in some vegetarians. Incapacitation of the body to absorb Vit-B₁₂ from intestines could lead to a type of anaemia known as pernicious anaemia. In addition, the mechanisms related to high serum cobalamin levels include three essential pathophysiological mechanisms exclude a direct increase in plasma Vit-B₁₂ by excess intake or administration. Firstly, a direct increase in plasma Vit-B₁₂ by increase of binding of Vit-B₁₂ to HCs. Secondly, an increase in TCB via excess production and finally, lack of clearance and a quantitative deficiency or lack of affinity of TCB for Vit-B₁₂^[6]. The reference interval of plasma Vit-B₁₂ according to international standards is 141-489 pg/ml.

On-the-other-hand, risks of solid cancer increase in individuals with high levels of Vit-B₁₂, generally higher than normal reference intervals^[1, 15]. Solid cancers refer to the growth of new cells, or alterations of morphological structures or disturbances of physiological operations, produced by an irregular growth of body tissues apart from blood, lymphatic cells and bone marrow. A solid cancer type is made of mass of irregular cells that stem from various tissues, including liver, breast, lung, and that fundamentally develop in the organs of their native cellular environment^[5]. In contrast, non-solid cancers are cancer kinds affecting the bloodstream, the bone marrow, and lymphatic nodes. Such cancers could drive from one of the two primary lineages of blood cell, namely myeloid lining and lymphoid cell lining. The myeloid cell lining usually generates erythrocytes, granulocytes, macrophages and mast and thrombocytes cells. On the other hand, the lymphoid cell lining creates B, NK, T and plasma cells could be origin of lymphomas and leukemias. Myeloma are produced from the bone marrow, whereas myelogenous leukaemiae either acute or chronic (AML, CML), a myeloproliferative expresses the principal health determinant in diseases are classified as myeloid in nature^[6].

The associations between high serum Vit-B₁₂ and solid tumours was first mentioned and documented^[7]. Several studies aimed to support and explain the practical diagnostic and prognostic implications of High serum cobalamin and solid tumours^[1]. The most frequent carcinomas are hepatocellular carcinoma^[8] and secondary liver tumours, colon cancer, breast cancer, cancer of the stomach and pancreatic tumours. In addition, high levels of plasma Vit-B₁₂ has been reported in patients with lung^[9], gastrointestinal and renal cancer^[10]. HC is synthesised by all of these tissues^[11]. The reasonable clarification for the high Vit-B₁₂ level is the increase of HC release to the circulation. In a study, patients with CML Chronic myeloid leukaemia have had an elevated Vit-B₁₂ levels, sometimes exceeding several thousand pmol/l^[12]. High Vit-B₁₂ and HC levels have also been described in polycythaemia vera, acute leukaemia, eosinophilic leukaemia and myeloproliferative neoplasm^[13]. Whereas the hypothesis for high Vit-B₁₂ level in CML is due to HC release from proliferating leukocytes. Although the current evidence is not as comprehensive, a recent paper documented that unexpected high Vit-B₁₂ levels had 4- to 18-fold higher risk of suffering from hepatic disease or haematological disease^{[14][15]}. In addition to solid tumour and haematological malignancies high serum Vit-B₁₂ have been associated to other diseases including renal diseases or liver diseases or during infection^[1].

In this work, we perform some initial experiments in order to validate and explore the role of Vit-B₁₂ in various types of cancer in our locality, such as solid and non-solid tumors. Firstly, we want to validate the significance of high serum Vit-B₁₂ level in cancer patients as compared with healthy individuals. Secondly, we examine if the extent of Vit-B₁₂ alterations in serum could be deployed in order to distinguish haematological cancers from solid tumour types, through a simple Vit-B₁₂ measurements.

Material and Methods

Sample collection and patients: Blood samples were obtained from cancer patients at Hiwa Haematology-Oncology Hospital, maintaining patient consents, in accordance with the ethical permission approval. We have excluded patient ages less than 18 years and older than 50 years. The control samples were obtained

from apparently healthy volunteers. Serum were separated by centrifugation at 2,000 rpm and then analysed. The serum samples (15 µl) were analyzed for *in vitro* quantitation of Vit-B₁₂ using Electrochemiluminescence immunoassay (ECLIA) on Cobas instrument (Elecsys®-Roche). The excess serums were kept frozen at - 20°C for later use.

Statistical Analysis: Statistical analyses were performed with SPSS program (version 20). Descriptive analysis was done with a table format, using proper charts and calculating percentages, ratios, means, median and standard deviations. Chi-square was used to find associations between categorical variables. The t-test for two independent samples was used to compare means and the P-value ≤ 0.05 was considered to be statistically significant.

Table 1. Overview representation of the Vit-B₁₂ levels in all patients. The data were grouped according to age, gender and type of cancer.

Category		No.	%	Plasma Vit-B ₁₂ levels		
				141- 489 pg/ml	489-800 pg/ml	>800 pg/ml
Age						
1	25-29	18	20.9	15	1	2
2	30-34	26	30.3	21	2	3
3	35-39	9	10.5	8	0	1
4	40-44	13	15.1	8	3	2
5	45-50	20	23.3	8	5	7
Gender						
1	Male	52	60.5	38	7	7
2	Female	34	39.5	23	4	7
Type of Cancer						
1	Solid	26	30.2	13	6	7
2	Non- Solid	24	27.9	12	5	7
3	Control	36	41.9	36	0	0
Total		86				

Table 2. Raw data of the statistical analysis, showing mean of difference and standard error

(I) Sample type	(J) Sample type	Mean Difference (I- J)	Std Error	Band	
				Lower	Upper
Solid Cancer	Non Solid Cancer	107.77	154.17	-277.14	492.69
	Control	469.78*	116.47	179.74	759.83
Non-Solid Cancer	Solid Cancer	-107.77	154.17	-492.69	277.14
	Control	362.01*	102.68	104.96	619.06
Control	Solid Cancer	-469.78*	116.47	-759.83	-179.74
	Non Solid Cancer	-362.01*	102.68	-619.06	-104.96
(*) The mean difference is significant at the 0.05 level					

Results and Discussion

This work was designed to check the Vit-B₁₂ status in newly diagnosed cancer patients at Hiwa Hospital in order to find and validate any possible relationship between various cancer types with serum Vit-B₁₂ levels. We asked also if the findings could have prognostic value according to the cancer group. Random blood samples of a total 86 individuals, including 50 cancer patients at various stages and types, were collected. The obtained data were analyzed according to cohort categories. As shown in (Table 1), the age

groups were subdivided into 5 cohorts. The majority of collected samples were in the age groups of 30-34 (30.4%) and 25-29 years (20.9%), where Vit-B₁₂ were in the normal range (141-489 pg/ml), apart from 5 samples which had higher level of Vit-B₁₂, (>800pg/ml). However, the age groups of 45-50 (23.3%), and 40-44 (15.1%) have had 9 samples, which had the highest Vit-B₁₂ range (>800pg/ml). Thus, the Vit-B₁₂ elevations are more pronounced in older group of patients (Table 1). In this study, we have subdivided the cancer type based on affected organ/tissues, into solid cancer (various body organs) and non-solid (hematopoietic) cancer.

Notably, we observed in all the 36 control samples the Vit-B₁₂ level was exclusively in the normal range (Table 1). However, the abnormally high values (>800 pg/ml) of Vit-B₁₂ levels, corresponding to non-solid cancer were 7 samples out of 24 samples (29 %). Similarly, the abnormal Vit-B₁₂ levels in solid cancer were 7 samples out of 26 samples (27%)(Table 1). Collectively, the data shows a clear elevation of abnormal levels of serum Vit-B₁₂ in solid and non-solid cancer in 25 patients out of 50 patients (50%), while in control group not a single case of elevated serum Vit-B₁₂ were observed (Table 1).

As illustrated in Figure 1 and Figure 2, the mean values of Vit-B₁₂ levels in both cancer types were much higher than controls. It is clearly shown that the distribution of Vit-B₁₂ values were scattered and much more heterogeneous in patient groups, while the control group had a homogenous range of Vit-B₁₂ (Figure 1 and 2). Collectively, the data were statistically significant presenting the differences between solid cancer ($p = 0.0005$), non-solid cancer ($p = 0.0005$) with control group (Figure 1 and 2). As indicated in Figure 1, we observe the standard errors in cancer samples were much higher, than control samples. This indicates that the Vit-B₁₂ levels had heterogeneous variations in all types of cancer (Figure 1). Probably, these variations and heterogeneity are more pronounced in solid tumor, compared to non-solid type. We could have been more confident if our patient samples were much greater numbers. However, this is our first pilot study to validate the Vit-B₁₂ biomarker assay in our locality. We aim for a comprehensive study with additional serum biomarker specifications along with Vit-B₁₂ assay.

Means Plots

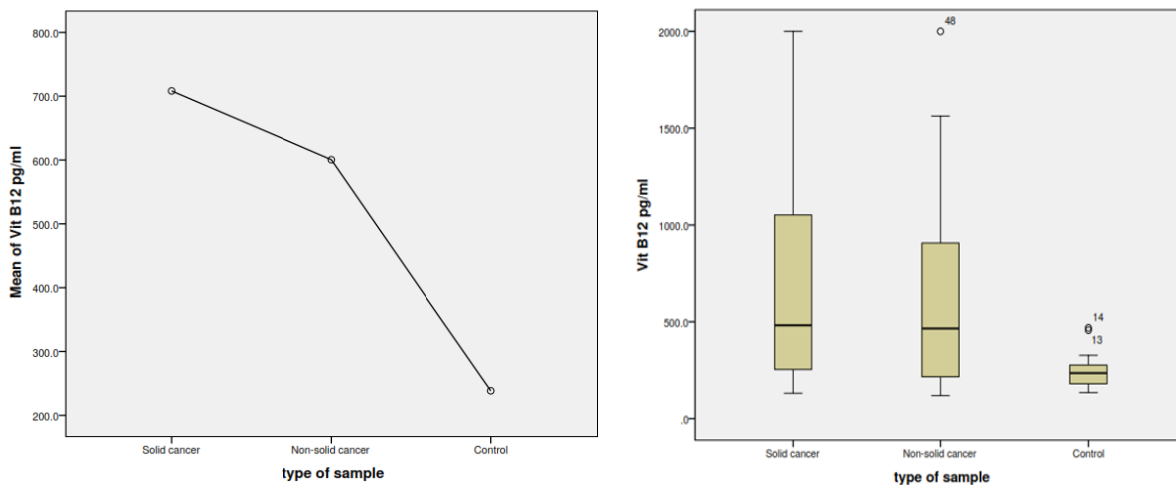


Figure 2. Plot of Vit-B₁₂ values in cancer types and Figure 1. Mean Vit-B₁₂ values in solid and non-solid cancer types compared to controls.

To illustrate further the differences between groups, the median Vit-B₁₂ values were calculated (Figure 2). The solid cancer median was 482 pg/ml, while in non-solid cancer was slightly lower 465.4pg/ml and the lowest were controls 235.2 pg/ml. The data clearly show a two-fold elevation of Vit-B₁₂ concentrations in cancer patients (Figure 2). In other words, both cancer groups (solid and non-solid) had at least two fold elevation above reference value of Vit-B₁₂ levels. In fact, 100% of the control samples were 100% compliance within the normal reference ranges (Figure 2). We believe that we do need further reference interval normalization or refinement, by selecting newly diagnosed cancer patients without drug treatment or any medications, in order to cover higher specificity of serum Vit-B₁₂ values, related to the cancer condition, clear from drug interference.

In addition, the upper most Vit-B₁₂ levels were observed in cancer patients, for solid cancer group was 1869 pg/ml (case 77) and for non-solid cancer was 1881 pg/ml (case 48), while for control group were much lower 334.6 pg/ml (case 13 and 14). Taken together, the significant differences between cancer cases and controls are highly represented in the plot data and also graphical means of Vit-B₁₂ levels, which indicated by showing median values and standard errors (Figure 1 and 2).

In Figure 3, the data of cancer cases were emerged in order to compare serum Vit-B₁₂ concentrations collectively with the control cases. Here again, we noticed the difference in the median values, of control samples 238.4 pg/ml much lower than the cancer cases 656.4 pg/ml. The spreading of Vit-B₁₂ values in cancer cases were much higher 150-1700 pg/ml, whereas in control samples about 150-350 pg/ml (Figure 3).

Conclusion

In this pilot research work, we have reported that high Vit-B₁₂ levels have been associated with the most life-threatening diseases, cancer. Nevertheless, our and other findings give rise to numerous unanswered questions and challenges. In general, the pathogenic backgrounds leading to high Vit-B₁₂ levels in the specific disease entities are yet to be scrutinized. Moreover, the performance of plasma Vit-B₁₂ as a marker for diseases other than Vit-B₁₂ deficiency has not been thoroughly evaluated.

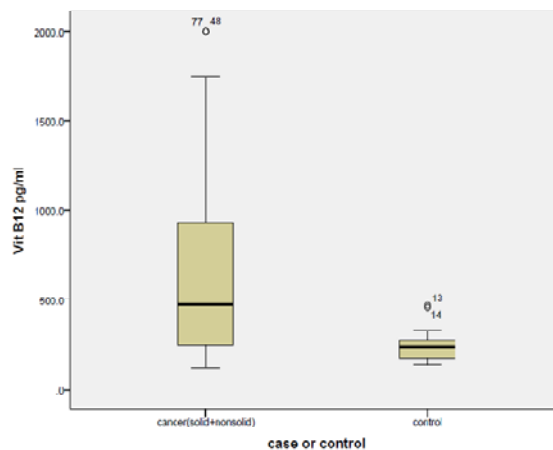


Figure 3. Comparison between emerging data of solid and non-solid cancer cases with controls.

In particular, we have explored that the risk of Vit-B₁₂ level elevation indicate the occurrence of cancer. In individuals with solid cancer type, the Vit-B₁₂ concentration in serum were between 600-800 pg/ml, whereas in individuals of non-solid cancer the values were between 500-700 pg/ml. Elevation of Vit-B₁₂ in cancer patient was significant compared to healthy individuals. Indeed, threshold value of Vit-B₁₂ between solid tumor types with hematological cancer is about 200 pg/ml difference. These were evident in plot and chart calculations that the significance value chosen were sufficient.

Still, we do not know the cause for heterogeneous distributions of free-serum Vit-B₁₂ concentration in cancer patients. Therefore, we want to study this phenomenon more closely in future. We are currently looking combinatorial markers with Vit-B₁₂, in order to increase the specificity and severity of various cancer types diagnosis and/or prognosis. Ideally, it will be of high interest to find another biochemical parameter(s), in order to perform more accurately permutations and diagnosis procedures that will be of benefit for health care system and patients.

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