ORIGINAL RESEARCH PAPER

Volume-7 | Issue-2 | February-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A STUDY OF PARIETAL CELL AND INTRINSIC FACTOR ANTIBODIES IN B12 DEFICIENCY ANAEMIAS: A NON-GASTROSCOPIC DESCRIPTIVE ANALYSIS IN A UNIVERSITY TEACHING HOSPITAL, INDIA

Clinical Research

Chandrasekhar Chodimella* — Associate Professor & Head, Department of Hematology, Sri Venkateswara Institute of Medical Sciences, Tirupati-517507, Andhra Pradesh, India *Corresponding Author

Pasupuleti Santhosh Kumar — Department of Biotechnology, Sri Venkateswara Institute of Medical Sciences, Tirupati-517507, Andhra Pradesh, India

Roopa Naik Sugali — Department of Endocrinology, Sri Venkateswara Institute of Medical Sciences, Tirupati-517507, Andhra Pradesh, India

Marthyaya Sumanth Kumar — Department of Hematology, Sri Venkateswara Institute of Medical Sciences, Tirupati-517507, Andhra Pradesh, India

Potukuchi Venkata Gurunadha Krishna Sarma — Department of Biotechnology, Sri Venkateswara Institute of Medical Sciences, Tirupati-517507, Andhra Pradesh, India

ABSTRACT

Background: The relationship between B12 deficiency and intrinsic factor antibody (IFA) and parietal cell antibody (PCA) was strongly established. A non-gastroscopic (non-invasive) analysis of the relationship is of great benefit.

Materials and methods: On the basis of serum B12, serum homocysteine, complete response to B12 therapy and BCSH guidelines, 120 patients were diagnosed to have IFA Positive Pernicious anemia (IFAPosPA) and IFA Negative Pernicious anemia (IFANegPA). The IFANegPA group was further divided into (PCA+ve) and (PCA-ve) subgroups. The IFA+ve(PCA+ve), IFA-ve (PCA+ve) and IFA-ve (PCA-ve) groups were studied with reference to the usual biomedical parameters.

Results: The mean serum B12 and homocystine levels were 180 pg/mL, 31 µmol/L respectively. 95% non-vegetarians, mean age 38 yrs, and female preponderance were observed. Serum homocysteine levels were normal in 21/120(17.5%) of patients in the background of sever iron deficiency. IFA+ve PA patients were 39/120 (32.5%), and IFA-ve PA group patients were 81/120 (67.5%), and patients with IFA-ve (PCA+ve) were 33/120 (27.5%). Patients with antibody positivity either IFA or only (PCA) were 72(60%), and IFA-ve (PCA-ve) (antibody negative) patients were 40%. There were significant differences in the B12, and homocysteine levels between antibody positive and negative groups.

Conclusion: 1) In the context of B12 deficiency, after excluding other non-immune causes a) IFA Pos PA patients showed more abnormal B12 and homocysteine levels than IFA Neg PA patients b) (PCA +ve) subgroup patients showed more significant B12 deficiency than (PCA-ve) patients , and both a,b may be indicating disease of longer duration or more severity. 2) PCA did not seem to have relevance in 72.5% of the cases with regards to the diagnosis of etiology of B12 deficiency or the diagnosis of PA.

KEYWORDS

B12 deficiency, Intrinsic Factor Antibody, Parietal Cell Antibody, Pernicious anaemia.

Introduction

There are many causes for cobalamin (Vitamin-B12) deficiency and pernicious anaemia (PA) with Intrinsic factor (IF) deficiency is one of them. PA is due to auto-immune mediated aetiology in which haematological, gastric and immunological alterations are seen in the form of Megaloblastic Anaemia, atrophic body gastritis (ABG), Parietal cell antibodies (PCA) and Intrinsic factor antibodies (IFA) [1]. This entity is found in all the continents [2,3] and probably is under diagnosed [4]. PCA, IFA were considered as the surrogate markers for the diagnosis of PA [5]. The diagnosis of PA is easily possible in the presence of IFA even though the absence of the same IFA does not rule out PA [6].

Cobalamin deficiency can be caused by [7]:

Infection- *H. Pylori*, Giardiasis Lamblia, fish tape worm.
Malabsorption: Pernicious Anemia, Coeliac disease, tropical sprue, Crohn's disease, Gastric resection; achlorhydria, PPI usage.
Pregnancy
Nutritional: Inadequate B12 intake, low cobalamin rich food, vegans
Infants / Children: Trans cobalamin deficiency, Imerslund-Grasbeck syndrome, other cobalamin mutations.

Guidelines aimed at the diagnosis of causes of cobalamin deficiency provide a pragmatic approach. These usually rely mostly on clinical judgement, in a given case [7]. The personal and family history of auto-immune diseases increases the pre-test probability of pernicious anaemia [1].

Parietal Cell Antibodies (PCA) are directed against the gastric enzyme H+/K+ - ATPase i.e. the proton pump which is secreted by the gastric parietal cells [8]. The atrophic gastritis is caused by the action of lymphocyte cluster of differentiation T-helper cell-1 inflammatory cells, directed against the β-subunit of this enzyme [9]. PCA are seen at a high frequency in PA (80-90%) particularly during the early stages and bind to both α and β sub units of gastric H+ / K+ ATPase. Circulating PCA belong to IgA, IgG and IgM isotypes. IgA, IgG antibodies are seen in the gastric juice [1].

The antibody reactivity to the α-unit includes the cytosolic side of the secretory membrane. The antigens in the β-sub unit are to be glycosylated, there by suggesting that the auto epitopes are situated in the luminal side to the glycoprotein. The localization of these molecules explains why the role of PCA in the pathogenesis of PA in vivo is elusive. In the later stages of the disease, PCA incidences comes down indicating the progression of the disease and loss of parietal cell mass and their antigens. In advanced cases the incidence is 55% [10]. PCA are not specific as they are also seen at low frequency in other auto immune disorders- like Hashimoto's thyroiditis or type-1 diabetes mellitus, elderly people even those without atrophic gastritis [1].

Intrinsic Factor is glycoprotein secreted by parietal cells. It has the role of high affinity binding to and transport of Vitamin B12. The IF-B12 complex is absorbed through the specific receptors in the terminal ileal epithelium and this function is interfered with by the IF antibodies [1].

In serum, type-1 (blocking antibody) reacts with Vitamin B12 binding site, and type-2 (binding of precipitating antibody) impedes the binding of IF to the ileal mucosal receptors [11]. Type-1 auto antibodies are seen in 70% and type-2 are found in 35-40% of PA patients. It is rare to have type-2 antibodies without type-1 antibody [1]. In the gastric juice IgA type IFA are found in 80% of patients with

PA, secreted by the infiltrating plasma cells of the mucosa. These bind to the residual IF and interferes with vitamin B12 absorption. Acidity enhances the binding of IF with vitamin B12 and achlorhydria caused by the loss of parietal cells favours the IFA+ vitamin B12 complexes.

IFA positivity is seen in 40-60% of patients with PA which can increase to 60-80% with the increasing duration of PA [12].

PCA can precede the clinical gastric disease by many years. The data on the predictive value of IFA for PA are not very conclusive. The production of IgA type of secretory antibodies in the stomach is seen as the auto immune gastritis gradually progresses to overt pernicious anemia. It is likely that IgA type IFA are required in the stomach to impair the absorption of B12. The serum IgG type IFA may not functionally correlate well with IgA type IFA secreted in the stomach [1]. Assessment of both PCA, IFA yield 73% sensitivity for PA and 100% specificity [5]. There are many studies providing adequate ground for the association between chronic/atrophic gastritis and the presence of IFA and PCA [13], but so far, only a few studies were done, particularly in India, a) on the patterns of IFA, PCA positivity and b) analyzing the relation between IFA, PCA and haematological parameters without gastroscopic interventions. Endoscopy is an invasive procedure and many B12 deficiency patients may be non-compliant due to the lack of signs and symptoms serious enough to subject themselves to endoscopy. BCSH guidelines 2014 subscribe to IFA test only for the diagnosis of pernicious anaemia, PCA was given no importance and not recommended as a diagnostic tool and endoscopy was not suggested in the algorithm. Pernicious anemias were divided into two groups – IFA PositivePA and IFA Negative PA on the basis of presence or absence of Intrinssic factor antibody. On the other hand Khan et al., 2009 advised IFA test to be done only if the PCA is positive. In the present study, the IFANegPA patients were studied with regards to various haematological parameters related to vitamin B12 deficiency or pernicious anemia.

Patients and Methods

This is a descriptive analysis on B12 deficiency patients. Out of 350 patients with Vitamin B12 deficiency 120 patients had the parietal cell antibodies and intrinsic antibodies assessment done. The study was cleared by the Institutional Ethics Committee (IEC.NO: 566). A written consent was obtained from all the patients studied.

All the patients of cobalamin deficiency were grouped with respect to presence of antibodies and the available data was analyzed to draw inference, i.e., a) IFA+ve, b) IFA-ve groups. Further, IFA-ve group was again divided into (PCA+ve) and (PCA-ve) subgroups. Theoretically, four groups could be considered -1) IFA+ve (PCA+ve), 2) IFA+ve (PCA-ve), 3) IFA-ve (PCA+ve) and 4) IFA-ve (PCA-ve). Since there were no patients with IFA+ve PCA -ve pattern, practically only three groups were possible.Haematological and biochemical parameters having relevance to cobalamin deficiency were compared among the groups.

Clinical investigations

Hemoglobin (Hb), hematocrit (Hct), red blood cell (RBC) indices, total white blood cell (TWBC) count, differential WBC count, Platelet count were measured using auto hematology analyzer BC-5300 (MINDRAY, Shenzhen, China), peripheral smear was done by using Leishman's stain. Liver function tests, renal function tests, thyroid function test, lipid profile, serum iron, total iron binding capacity (TIBC) were measured by using (Beckman ante analyzer DXC 600). Serum ferritin (Chem well analyzer), ultra-sonography abdomen, X-ray chest, intrinsic factor antibody (IFA) (by Immunofluorescence), parietal cell antibody (PCA) (by Immunofluorescence), vitamine B12, serum homocysteine (iced sample), serum folic acid and RBC folate levels were assessed by Carbonylmetalloimmunoassay (CMIA).

Investigative pathway

I. Vitamin B12 deficiency was considered on the basis of

a) Serum vitamin B12 levels <300 pg/ml [14, 15]
b) Complete Blood counts (CBC), Peripheral blood smear, Morphology of WBC and RBC and Platelets
c) S. Homocysteine levels >14 µmol/ml
d) Complete response in the clinical and hematological parameters with vitamin B12 treatment in 30-45 days.
e) Ruling out Folic acid deficiency (normal -serum Folic acid >3.1 ng/ml Red blood cell folate levels >243 ng/ml.)

II. Associated Iron deficiency was treated with oral Iron therapy. Iron deficiency was considered on the basis of

a) MCV is less than 100 fl
b) Serum Ferritin levels < 40 ng/ml
c) Serum Transferin saturation <30%
d) MCV < 80fl after the restoration of Hb to more than 13 gm.

III. Other causes like anemia of chronic systemic diseases, hemoglobinopathy are ruled out

IV. Investigating the cause for B12 deficiency.

- Systemic, GI tract causes, drugs like Levodopa, Metformin, PPI, Oral contraceptives
- PCA, IFA antibody testing was done before administering the first B12 injection, or minimum more than 8 days after the injection.
- MMA levels (methyl melanoic acid), Gastric mucosal Biopsy and *H. Pylori* infection were not tested for.
- Food consisting of minimum 3 eggs per week was considered as non-vegetarian diet.

Statistical analysis

The association between two categorical variables (patients and healthy subjects) was evaluated by Chi-square (χ^2) test, a p-value <0.05 was considered as significant by using statistical software IBM SPSS, version 20 (IBM SPSS statistics, Somers NY, USA).

Results and Discussion

Out of 120 patients, 114 (95%) were non vegetarians, IFA were positive in 32.5% (IFAPosPA) and 67.5% patients could be considered as IFANegPA as per BCSH Guidelines [7] (Table 1). The results in our study indicated that 32.5% (39/120) of patients were IFA +ve and 67.5% (81/120) were IFA –ve. Either PCA or IFA were seen in 60% (72/120) of our patients while 40% (48/120) showed neither of them (Table. 1). Earlier Indian studies showed very low prevalence of IFA positivity [19]. In our study, PCA was negative or not found in 40% of the patients and ,it's role was anyway insignificant (even if it was positive) where IFA was positive (32.5%). Therefore, in nearly 72.5% of our patients with B12 deficiency there was no significant role for PCA incorporate [7]. The prevalence of various groups with respect to the presence or absence of IFA, PCA antibodies, in our study were comparable to earlier studies (Table 1). In our study, 32.5% patients with documented B12 deficiency had IFA suggesting Pernicious anaemia as per the classic definition. According to Loukili et al, 2004 [17], PA accounted for 20-50% of B12 deficiency patients. PA was seen in 15-25% of B12 deficiency in elderly people [25]. In Chinese people PA was seen 61% patients with B12 deficiency / Folate deficiency [26].

Overall, the mean serum cobalamin levels were 180 pg/ml and the 50th percentile and 75th percentile levels were 155 pg/ml and 226 pg/ml respectively. Earlier studies showed comparable results, 257.6±79.4 pg/ml (Mahmoud et.al) [16], 73 pg/ml (20-1960) (Loukili et al) [17], 127.8±114 pg/ml (Amarapurkar et al) [18] and 53.9 pg/ml (12.5 -200) (Desai et al) [19]. The mean and median transferrin saturation were 18.05% and 8.95% respectively suggesting of definite iron deficiency in our patients (Table 2) S. Homocysteine levels were high in most (82.5%) of our patients, incorporate mean 31 (7.7-94.5) µmol/L, median 22.5 µmol/L. In IFA positive patients, none had homocysteine less than normal levels, where as some IFA negative patients (21/81, 25.9% or overall 21/120 17.5%) showed microcytic picture (62-87 fl, mean 69.7, median 69 fl,) low transferrin saturation (2-39%, mean 11.%, median 3.9%, 75th percentile 9%), low ferritin (2.16-101 ng/ml, mean 20.3, median 3.86, 75th percentile 7.3), normal homocysteine levels (7.7-13.5 µmol/l, mean 11.2, median 12.3 , 75th percentile 12.8), low to borderline normal B12 levels (115-486 pg/ml, mean 265, median 245, 75th percentile 328) and a good response only when B12 was added to the oral iron therapy suggesting that normal homocysteine levels may not rule out vitamin B12 deficiency, particularly in the presence of iron deficiency [20-22]. This was reflected in para e), f). However, three patients (from the neighbouring states Tamil Naidu) of 21 in IFA-ve group had low mean MCV 64fl, normal transferrin saturation 39%, and ferritin101 ng, levels and with low B12 levels 161 pg/ml, but with normal homocysteine levels 7.7 µmol, who, after repleting with iron and B12, were later diagnosed to have thalassemia trait. Normal iron stores in B12 deficiency could be attributed to iron deficiency. Only two patients had low serum folic acid with normal RBC folate levels.

The age group (38.5 yrs) in our study is much younger than that in the western countries, 74 Years (25-93) (Loukili et al) [17] 59.6 years +/-

(Carmel et al) [23], 46.5 years +/- 17.5 years (Mahmoud et al) [16]. In Indians, the average age was 30.74 years (Desai et al) [19] and 38.48 years (our study) (Table 2). No significant difference in the age of the patients among the groups with and without IFA was seen.

Overall, there was slight female preponderance in our study, M:F = 1:1.3 and it was 1:3.3 in the IFA +ve group (Tables 2, 3). The patients of Pernicious Anaemia in the western countries showed much more female dominance. Studies from Denmark showed the prevalence of PA to 1.7% in females and 0.8% in males [24].

All IFA positive PA patients had PCA positivity. IFA negative PA patients were incorporate PCA+ve in 33/81, 40.7% (PCA+ve subgroup) and PCA-ve in 48/81 59.3% (PCA-ve subgroup). IFA+ve (PCA +ve) group, when compared with IFA-ve (PCA-ve) subgroup, showed significantly lower serum cobalamin levels (Table 3). Similar observation was made with IFA+ve PCA+ve patients when this group was compared to both IFA-ve, (PCA-ve) subgroup and IFA-ve (PCA+ve) subgroup patients put together (Table 4). It simply meant that the BCSH entities, IFA Pos PA patients were showing lower levels cobalamin (Cbl) than IFANegPA patients. This is consistent with the observations of Mahmoud et al [16]. However, the earlier studies by Amarapurkar et al [18], Loukili et al [17], Khan et al [27] where the diagnosis was resting only on serum B12 levels, showed no difference in the serum cobalamin levels with respect to the presence or absence of antibodies. We are not sure whether this difference could be related to the selection criteria of B12 deficiency between our study and theirs. The diagnosis of B12 deficiency in our study was on the basis of serum homocysteine levels and clinical response to B12 therapy, instead of relying only on serum B12 levels [9]. In addition, the cut-off value of serum B12 levels was much higher in our study than that in other studies [16-18, 27]. Lower B12 levels in anemia might indicate a more longstanding disease.

All patients in our study were B12 deficient as per the selection criteria. Comparison of haematological and biochemical parameters among the groups was to know the importance of the association between the presence of the antibodies and the pathophisiology of B12 deficiency.

a) IFA+ve PCA+ve (two antibodies positive) group vs IFA-ve (PCA-ve) subgroup (antibody negative): the antibody positive group had significantly lower B12 levels, higher homocysteine levels and lower platelets count (attributable probably to B12 deficiency). The normocytic picture and the lower than normal transferrin saturation levels were comparable in both the groups and suggested an associated and underlying iron deficiency (Table 3).
b) IFA+ve PA group vs IFA-ve PA group: IFA+ve patients had significantly lower B12, platelet count and transferrin saturation levels but surprisingly comparable homocysteine levels or disproportionate rise in homocysteine levels for the given B12 levlels.(table 4) . This aspect was also reflected in morphological groups like microcytic and normocytic groups, paragraphs e) and f).
c) IFA+ve PCA+ve (two antibodies positive) group vs IFA-ve (PCA+ve) (only PCA positive) subgroup: the serum B12 and homocystin levels and platelets count in both the groups .were comparable without significant difference suggesting that PCA also could serve as a good marker for B12 malabsorbtion but not for iron absorption but the important observation was the homocysteine levels were disproportionately low for the given B12 levels in the IFA+ve PCA group. This group of patients showed significantly lower transferrin saturation and also female preponderance.(Table 5).
d) IFA-ve (PCA+ve) (only PCA positive) subgroup vs IFA-ve (PCA-ve) (antibody negative) subgroup: the Hb, PCV and serum B12 levels were significantly lower in PCA+ve subgroup. The higher homocystin levels in PCA+ve subgroup would serve as a sensitive marker for relatively higher cellular B12 deficiency. Transferrin saturation is lower in PCA-ve subgroups and again there is a female preponderance (Table 6). This observation of lower iron stores can possibly but partly be explained by the understanding that the PCA disappear as the gastric mucosal inflammation progresses to atrophic gastritisc [10,12] causing iron deficiency.

The morphological aspects of haematological parameters were due to the combined effects of various nutrient deficiencies eg., vitamin B12, iron, folic acid etc. Microcytic picture of peripheral blood smear in our study, showed predominatly iron deficiency and macrocytic picture was suggestive of predominant cobalimin deficiency. From microcytic to macrocytic pictures, there was a stepwise fall in serum B12 levels from 224±106 µg/mL to 133±51 µg/mL (Table 7). Conversely, in the same order the tranferrin saturation, serum feritin, S. homocystine levels showed regular stepwise rise [28]. This indicates relative sparing of iron stores in B12 deficiency states. The morphological groups-microcytic, normocytic, macrocytic groups did not show any significant relationship to the presence or absence of PCA antibodies among the IFA-ve PA group patients. Most of the normocytic patients were IFA +ve PA (69.2%) suggesting significant deficiency of both B12 and iron (combined deficiency) whereas the microcytic (predominant iron deficiency) and the macrocytic (predominant B12 deficiency) groups showed IFA positivity in a few patients, 16.6% and 11.1% respectively. The B12 levels were comparable in the normocytic and the macrocytic group but the B12 deficiency associated with significant iron deficiency was responsible for the normocytic morphology in the 'normocytic -IFA+ve ' group. This might lend support to the notion that IFA positivity increases as the disease progresses to atrophic gastritis thereby causing both iron and B12 deficiency [10,12].

e) Microcytic vs normocytic groups: microcytic group showed relatively higher B12 levels, lower homocysteine levels (mild B12 deficiencies) and higher platelets counts and lower ferritin levels, the differences being statistically significant. In both the groups, serum homocysteine levels were increased but the increase is significantly less in the microcytic group and in nearly 18/54(33%) of this group, homocysteine levels were normal, it's mean being 11.1 µmol. [24, 28] (Table 8).
f) Normocytic vs macrocytic group: the serum homocysteine and LDH levels, MCV and MCH, were significantly higher in the macrocytic group ($p<0.001$) for the given (comparable) B12 levels. Similarly macrocytic group showed higher ferritin/ transferring saturation levels indicating pure B12 deficiency and adequate or excess iron reserves. Relatively lower homocysteine, and lower transferrin saturation (associated iron deficiency) were seen in normocytic group (Table 9). It is interesting to see the homocysteine levels, a sensitive marker for cellular B12 deficiency, were lower in the normocytic group when the availability of B12 in terms of serum levels (comparable B12 deficiency) was similar in both the groups. Whether associated iron deficiency had a role in keeping homocysteine levels lower than expected in B12 deficiency , as suggested by a few studies needs to be verified [20,21,22,].
g) Microcytic vs macrocytic groups: microcytic group showed relatively higher B12 levels (mild B12 deficiency), and platelet counts and lower homocystein levels and iron stores indicating predominant iron deficiency. On the other hand macrocytic group demonstrated pure B12 deficiency .The possible explanation could be-all are B12 deficient as evidenced by homocystein levels also, and the associated ongoing or pre-existing iron deficiency was the factor responsible for the resultant difference in the morphology (MCV). Therefore, milder deficiencies of B12 with pre-existing significant iron deficiency (combined deficiency) and significant pure B12 deficiency must have led to microcytic and macrocytic pictures at Hb of 8 gm, respectively.

Transferin saturation was significantly lower in the IFA+ve and PCA+ve groups than that in the IFA–ve, (PCA–ve), IFA –ve (PCA +ve) subgroups individually and also both put together (Tables. 3, 4). This supports concept that the IFA appear after the complete progression of chronic gastritis to atrophic gastritis [10,12].

The association with thyroid disease in our study was 12/39, 30.7% (IFA +ve, PCA +ve), 3/33, 9% (PCA +ve, IFA –ve) and 6/48, 12.5% in (IFA –ve, PCA-ve) groups [19, 28-31]. Vitiligo, Hypoadrenalism, Hypothyroidism- all together were seen in one patient only suggesting Autoimmune Polyendocrine syndrome (APS). None was diabetic. IFA, PCA could be associated with H. Pylori infection [5, 13, 16, 28, 32, 33], though the relation was not yet settled. It was concluded that H. Pylori gastritis represented the early phase of infectious process which would later be gradually replaced by an auto immune mechanism, terminating in the irreversible destruction of gastric mucosa [28]. Therefore all the patients with B12, iron deficiency with or without IFA, PCA, should be looked for H. Pylori infection, considering the high prevalence of H. Pylori in India [34, 35]. Eradication of H. Pylori was included in the “seek and eradicate” Paradigm to cure the disease [5].

The limitation in our study was the lack of data on gastric mucosal biopsy features and H. pylori status of our patients. In IFA+ve patients the symptoms and signs did not show specific indication for an invasive procedure like gastroscopy. In the absence of abdominal symptoms and chronic usage of PPIs, the gastric mucosal biopsy would only confirm the presence of gastric inflammation and its nature and possible etiology whether Type A or B ,in IFA positive patients [5]. It is well known that it is the auto immune mechanism, through IFA / PCA that causes atrophic gastritis resulting in reduced intrinsic factor and B12 absorption. There is a huge pile of evidence to consider IFA (100%), PCA (91%) as highly specific for atrophic gastritis [13, 18]. Therefore, in the context of B12 deficiency, the presence of IFA and PCA does lend support, indirectly but reliably, to an auto immune mechanism responsible for B12 deficiency. The gastric mucosal biopsy however, does not entirely rule in or rule out either Type A, autoimmunegastritis (ABG) (antrum spared) or H. Pylori related immune mediated gastritis (Type B) (antral involvement) since there could be an overlap of both features in nearly 27% of the patients with IFA and PCA positivity [13]. Therefore, in the absence of IFA and PCA positivity gastric mucosal biopsy becomes crucial with respect to the diagnosis of etiology of B12 deficiency, particularly in differentiating the simple atrophic gastritis with achlorhydria and defective food-cobalamine dissociation causing cobalamine malabsorption [6]. Since IFA and PCA have been considered surrogate markers for autoimmune pathology (Pernicious anaemia), the limitation of our study has been to its inability to diagnose the aetiology of the B12 deficiency in IFA –ve groups of patients with certainty. However, secretory IF estimation through intra gastric tubage after pentagastrin stimulation could establish the deficiency of intrinsic factor and thereby confirms Pernicious anaemia [6]. It may be beneficial to consider gastric mucosal biopsy after correcting the haematological parameters and GI tract mucosal changes secondary to B12, folic acid, iron deficiency, so that the primary nature of the mucosal pathology is established.

Conclusions

1. The B12 deficiency anaemia patients in our study were 95% non-vegetarians, comparatively younger in age group, mean B12 and homocysteine levels were 180 pg/ml and 31 µmol/L respectively.
2. Serum homocysteine was elevated in 82.5% of the patients. It was normal in 17.5% with definite B12 deficiency in association with severe iron deficiency. This observation could not be explained biochemically or pathophysiologically with regard to requirement of B12 and iron at different stages of erythroid maturation.
3. IFA+ve PA were 32.5% and IFA-ve PA were 67.5%. More severe B12 and iron deficiencies were noted in IFA+ve PA group probably suggesting more longstanding disease.
4. PCA had no role in 72.5% of the patients in the work up of B12 deficiency. It was observed that B12 levels in IFA-ve PCA+ subgroup of patients were lower than IFA-ve PCA-ve subgroup patients and compar able to IFA+ve PA patient.

Since we cannot be sure of deficiency status of the laboratory controls, more studies with regard to the assessment of normal cut off values in the healthy people are required particularly of homocysteine (checking the values again after the standard and full doses of B12 or folic acid) so that comparative studies can be done among the groups.

Conflict of interest

All authors declare that there is no conflict of interest.

Acknowledgements

We sincerely acknowledge Sri Venkateswara Institute of Medical Sciences and University, Tirupati, Andhra Pradesh, India for providing facilities to carry out this work.

Table: 1. The frequencies of IFA and PCA in conditions like chronic gastritis with B12 deficiencies

S. No	Author	IFA +ve PCA +ve (a)	IFA –ve PCA +ve (b)	IFA –ve PCA –ve (c)	(1) IFA –ve PCA+ve (2) IFA -ve PCA –ve (b+c)
1	Carmel et al[20]	42%	13%	15%	
2	Amarapurkar et al[18]	32%	48%	16%	64%
3	Mahamoud et al [16]	18.50%	9%		
4	Desai et al[19]	15%	71%		
5	Our study (n=120)	32.50% (n=39)	27.50% (n=33)	40% (n=48)	67.50% (33/81)

Table: 2. Overall values of 120 patients of B12 deficiency

Parameters	Range	Mean	S.D	Median
Age (yrs)	15-69	38.475	14.5479	39.5
Hb (g/dl)	4.6-15	8.9175	2.55131	8.4
PCV (%)	13.8-44	29.5325	7.48436	28.9
MCV (fl)	56-123	85.1	18.7313	85.5
Platelets (10^9/L)	0.7-5.8	2.727	1.07658	2.82
S. B12 (pg/mL)*	59-486	180.75	89.1253	155
S. Homocystine (µmol/L)#	7.7-94.5	31.159	18.9537	22.5
S. folic acid (ng/mL)	1-31.5	13.309	9.64621	10.23
RBC folate (ng/mL)	135-1993	615.55	333.549	549.5
Transferrin Saturation (%)	1.1-111	18.055	21.3663	8.95
S. Ferritin (ng/mL)	1.3-521	72.537	106.423	15.2
S. LDH (U/L)	237-11335	971.85	1871.37	450

Sex (M:F) (45M, 75F, 1:1.7)
* S. B12 levels- 90th percentile=276 pg/mL, 95th percentile=328 pg/mL, 99th percentile=418 pg/mL
>300 pg/mL = 12 patients; 200-300 pg/mL = 21 patients

S. Homocystine- 5th percentile=14.25 µmol/L; 25th percentile=17 µmol/L
<14.25 µmol/L = 21 patients

Table: 3. Comparison between IFA+ve, PCA+ve group and IFA-ve, PCA-ve group

	IFA+ve, PCA+ve	IFA-ve, PCA-ve	
Parameters	Mean ± SD	Mean ± SD	P value
	N = 39	N = 48	
Age	39.23 ± 14.885	40.25 ± 13.400	0.738
Sex (male/female)	9/30 (1:3.3)	18/30 (1:1.7)	
Hb (g/dl)	9.262 ±2.507	9.350 ± 2.457	0.869
PCV (%)	31.00 ± 8.095	30.64 ± 6.700	0.823
MCV (fl)	88.62 ± 11.066	82.19 ± 20.404	0.081
MCH	27.23 ± 5.508	25.19 ± 7.394	0.156
MCHC	29.85 ± 2.889	29.56 ± 3.814	0.702
TWBC (10^3/L)	6215.38 ± 2159.988	5837.50 ± 1848.25	0.382

*Platelets (10^9/L)	2.423 ± 0.8904	2.892 ± 0.9321	0.019*
*S. B12 (pg/mL)	142.31 ± 44.641	219.25 ± 95.849	0.001*
*S. Folic Acid (ng/mL)	8.112 ± 6.1434	14.362 ± 8.962	0.001*
*RBC Folate (ng/mL)	500.00 ± 102.57	749.25 ± 437.423	0.001*
*S. Homocysteine (µmol/L)	33.54 ± 14.336	24.37 ± 16.539	0.008*
S. Iron (µg/dl)	45.23 ± 29.225	52.94 ± 48.986	0.39
TIBC (µg/dl)	384.77 ± 74.883	396.56 ± 92.679	0.522
Transferrin Saturation (%)	12.238 ± 8.116	15.712 ± 15.240	0.203
S. Ferritin (ng/mL)	56.862 ± 84.706	56.886 ± 72.943	0.999
S. LDH (U/L)	835.85 ± 1265.370	719.12 ± 575.533	0.569
Values are the mean ±SD from three determinations			

Table: 4. Comparison between IFA+ve and IFA-ve regardless PCA positivity

Group	**IFA +ve (n=39)**	**IFA –ve (n= 81)**	**P value**
Parameters	**Mean ± SD**	**Mean ± SD**	
Age	39.23±15.2	38.11±14.4	0.84
Sex(male/female)	9/30 (1:3.3)	36/45 (1:1.3)	
Hb (g/dl)	9.26±2.5	8.75±2.5	0.3
PCV (%)	31±8.3	28.83±7.1	0.13
MCV (fl)	88.62±11.3	83.41±21	0.15
MCH	27.23±5.6	25.59±8.0	0.25
MCHC	29.85±2.9	29.59±3.5	0.69
TWBC (10^3/L)	6215±2219	6266±2119	0.9
*Platelets (10^9/L)	2.42±0.9	2.87±1.1	0.03*
*S. B12 (pg/mL)	142.31±45.8	199±99	0.001*
*S. Folic Acid (ng/mL)	8.11±6.31	15.81±9.6	0.001*
*RBC Folate (ng/mL)	500±105	671±389	0.007*
S. Homocystine (µmol/L)	33.54±14	30.0±20	0.33
S. Iron (µg/dl)	45.23±30	59.19±57.3	0.15
TIBC (µg/dl)	384±76	378±125	0.75
*Transferrin Saturation (%)	12.23±8.3	20.85±25	0.03*
S. Ferritin (ng/mL)	56.86±87	80.0±115	0.26
S. LDH (U/L)	835±1300	1037±2111	0.58
Values are the mean ±SD from three determinations			

Table: 5. Comparison between IFA+ve, PCA+ve group and IFA-ve, PCA+ve group

	IFA+ve, PCA+ve	IFA-ve, PCA+ve	
Parameters	Mean ± SD	Mean ± SD	P value
	N = 39	N = 33	
Age	39.23 ± 14.885	35.00 ± 15.137	0.237
Sex (male/female)	9/30 (1:1.3)	18/15 (1.2:1)	
Hb (g/dl)	9.262 ±2.507	7.882 ± 2.435	0.021
*PCV (%)	31.00 ± 8.095	26.18 ± 6.709	0.008*
MCV (fl)	88.62 ± 11.066	85.18 ± 22.319	0.4
MCH	27.23 ± 5.508	26.18 ± 8.883	0.543
MCHC	29.85 ± 2.889	29.64 ± 2.977	0.763
TWBC (10^3/L)	6215.38 ± 2159.988	6890.91 ± 2291.47	0.203
Platelets (10^9/L)	2.423 ± 0.8904	2.845 ± 1.3608	0.119
S. B12 (pg/mL)	142.31 ± 44.641	170.18 ± 95.096	0.107
*S. Folic Acid (ng/mL)	8.112 ± 6.144	17.918 ± 10.097	0.001*
RBC Folate (ng/mL)	500.00 ± 102.567	557.64 ± 258.282	0.205
S. Homocysteine (µmol/L)	33.54 ± 14.336	38.23 ± 23.238	0.299
S. Iron (µg/dl)	45.23 ± 29.225	68.27 ± 65.876	0.053
TIBC (µg/dl)	384.77 ± 74.883	351.00 ± 155.863	0.234
*Transferrin saturation (%)	12.238 ± 8.116	28.336 ± 33.0350	0.04*
S. Ferritin (ng/mL)	56.862 ± 84.706	113.826 ± 150.499	0.047
S. LDH (U/L)	835.85 ± 1265.370	1500.18 ± 3163.90	0.233
Values are the mean ±SD from three determinations			

Table: 6. Comparison between IFA-ve PCA+ve (one antibody +ve) and IFA-ve PCA-ve (antibody negative) groups

Group	IFA-ve PCA+ve	IFA-ve PCA-ve	
Parameters	Mean ± SD	Mean ± SD	P value
	n=33	n=48	
Age	35±15.14	40.25±13.40	0.104
Sex(male/female)	18/15	18/30	
*Hb (g/dl)	7.88±2.44	9.35±2.46	0.01*
*PCV (%)	26.18±6.71	30.64±6.70	0.004*
MCV (fl)	85.18±22.32	82.19±20.40	0.534
MCH	26.18±8.88	25.19±7.39	0.586
MCHC	29.64±2.98	29.56±3.81	0.926
*TWBC (10^3/L)	6890.91±2291.47	5837.50±1848.25	0.025*
Platelets (10^9/L)	2.85±1.36	2.89±0.93	0.854
*S. B12 (pg/mL)	170.18±95.09	219.25±95.85	0.026*

S. Folic Acid (ng/mL)	17.92±10.10	14.36±8.96	0.1
*RBC Folate (ng/mL)	557.64±258.28	749.25±347.42	0.027*
*S. Homocystine (µmol/L)	38.23±23.24	24.36±16.54	0.002*
S. Iron (µg/dl)	68.27±65.88	52.94±48.99	0.233
TIBC (µg/dl)	351.00±155.86	396.56±92.68	0.103
*Transferrin Saturation (%)	28.34±33.04	15.71±15.24	0.023*
*S. Ferritin (ng/mL)	113.83±150.50	56.89±72.94	0.026*
S. LDH (U/L)	1500.18±3163.89	719.12±575.53	0.098

Table: 7. Comparison among the red blood cell morphological groups

MCV	IFA+ve %	PCA+ve %	IFA-ve %	PCA-ve %	IFA-ve/ PCA-ve%	Serum B12	Transferrin Saturation	S. Ferritin	S. Hcy (µmol/L)	Platelets
Microcytic picture	16.6	50	83.3	50	50	224.44±107.95	11.64±15.69	30.34±58	22.76±13.50	3.37±0.853
Normocytic picture	69.2	76.9	30.7	23	23	153.15±44.80	16.27±10.17	66.38±91.9	28.88±14.71	2.37±0.89
Macrocytic picture	11.1	55.5	88.8	44.4	44.4	133.22±51.4	33.47±34.38	165.8±137	51.25±20.4	1.95±1.05

Table: 8. Comparison according to mean corpuscular volume (MCV)

Parameters	Microcytic (<79 fl) Mean ± SD n=54	Normocytic (80-99 fl) Mean ± SD n=39	P value
*Age Sex (M:F)	42.72±10.54 18/36 (1:2)	33.92±14.22 12/27 (1:2.2)	0.001*
*Hb (g/dl)	8.03±1.61	10.65±2.72	0.001*
*PCV (%)	27.84±3.95	33.77±8.7	0.001*
*MCV (fl)	67.83±5.78	90.15±4.86	0.001*
*MCH	19.39±2.83	28.77±3.3	0.001*
*MCHC	28.22±2.74	31.31±2.61	0.001*
TWBC (10^3/L)	6700±1866	6153±1980	0.178
*Platelets (10^9/L)	3.37±0.83	2.37±0.86	0.001*
*Serum B12 (pg/mL)	224.44±105.9	153.15±43.6	0.001*
*S. Folic Acid (ng/mL)	14.44±8.76	9.42±7.77	0.005*
*RBC Folate (ng/mL)	663.9±359	542.8±253	0.074
*S. Homocystine (µmol/L)	22.76±13.24	28.88±14.3	0.036*
S. Iron (µg/dl)	44±52	57.2±29.9	0.158
*TIBC (µg/dl)	452.5±100	367.69±56	0.001*
Transferrin saturation (%)	11.63±15.3	16.26±9.9	0.103
S. Ferritin (ng/mL)	30.34±58	66.38±91.9	0.023*
S. LDH (U/L)	493.1±187	820±1269	0.65
PCA+ve, (-ve) (%)	50, (50)	77, (23)	
IFA+ve, (-ve) (%)	17, (83)	69, (31)	
Values are the mean ±SD from three determinations			

Table: 9. Comparison according to mean corpuscular volume (MCV)

Parameters	Normocytic (80-99 fl) Mean ± SD n=39	Macrocytic (>100 fl) Mean ± SD N=27	P value
Age Sex (M:F)	33.92±14.22 12/27 (1:2.2)	36.56±18.48 15/12 (1.2:1)	0.521
*Hb (g/dl)	10.65±2.72	8.16±2.52	0.001*
*PCV (%)	33.77±8.7	26.79±8.29	0.002*
*MCV (fl)	90.15±4.86	112.33±6.96	0.001*
*MCH	28.77±3.3	35.78±3.14	0.001*
MCHC	31.31±2.61	30.22±3.92	0.182
TWBC (10^3/L)	6153±1980	5488±2534	0.236
Platelets (10^9/L)	2.37±0.86	1.94±1.00	0.071
Serum B12 (pg/mL)	153.15±43.6	133.22±51.4	0.095
S. Folic Acid (ng/mL)	9.42±7.77	16.65±10.65	0.002
RBC Folate (ng/mL)	542.8±253	623.7±362	0.289
*S. Homocystine (µmol/L)	28.88±14.3	51.25±19.64	0.001*
S. Iron (µg/dl)	57.2±29.9	72.2±62.2	0.197
*TIBC (µg/dl)	367.69±56	253.6±48.5	0.001*
*Transferrin saturation (%)	16.26±9.9	33.46±33.0	0.003*
*S. Ferritin (ng/mL)	66.38±91.9	165.8±137	0.001*
*S. LDH (U/L)	820±1269	2148±3374	0.028*
PCA+ve, (-ve) (%)	77, (23)	56, (44)	
IFA+ve, (-ve) (%)	69, (31)	11, (89)	
Values are the mean ±SD from three determinations			

Table: 10. Comparison according to mean corpuscular volume (MCV)

Parameters	Microcytic (<79 fl) Mean ± SD n=54	Macrocytic (>100 fl) Mean ± SD N=27	P value
Age	42.72±10.54	36.56±18.48	0.063
Hb (g/dl)	8.03±1.61	8.16±2.52	0.783
PCV (%)	27.84±3.95	26.79±8.29	0.439
MCV (fl)	67.83±5.78	112.33±6.96	0.001*
MCH	19.39±2.83	35.78±3.14	0.001*
MCHC	28.22±2.74	30.22±3.92	0.009*
TWBC (10^3/L)	6700±1866	5488±2534	0.017
Platelets (10^9/L)	3.37±0.83	1.94±1.00	0.001*
Serum B12 (pg/mL)	224.44±105.9	133.22±51.4	0.001*
S. Folic Acid (ng/mL)	14.44±8.76	16.65±10.65	0.322
RBC Folate (ng/mL)	663.9±359	623.7±362	0.637
S. Homocystine (µmol/L)	22.76±13.24	51.25±19.64	0.001*
S. Iron (µg/dl)	44±52	72.2±62.2	0.035*
TIBC (µg/dl)	452.5±100	253.6±48.5	0.001*
SF (%)	11.63±15.3	33.46±33.0	0.001*
S. Ferritin (ng/mL)	30.34±58	165.8±137	0.001*
S. LDH (U/L)	493.1±187	2148±3374	0.001*
PCA+ve, (-ve) (%)	50, (50)	56, (44)	
IFA+ve, (-ve) (%)	17, (83)	11, (89)	
Values are the mean ±SD from three determinations			

References

1. Bizaro N, Antico A. Diagnosis and classification of pernicious anemia. Autoimmunity Reviews. 2014;13(4):565-568.
2. Maktouf C, Behir A, Louzir H, et al. Megaloblastic anemia in North – Africa. Haematologica. 2006;9:990-1.
3. Week MN, Brenner H. Prevalence of chronic atrophic gastritis in different parts of the word. Cancer Epidemiol Biomarkers Prev. 2006;15:1083-94.
4. Carmel R. Prevalence of undiagnosed pernicious anemia in the elderly. Arch Intern Med. 1996;156:1097-1100.
5. Lahner E, Annibale B, pernicious anema: New insight from gastroentero world J Gastroenterol. 2009;15(41):5121-5128.
6. Cattan D. Pernicious anemia; what are the actual diagnosis criteria? World J Gastroenterol. 2011;17(4):543-44.
7. Davalia V, Hamilton MS, Molloy AM. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. Br J Haematol. 2014;166(4):496-513.
8. Toh BH, Sentry JW, Alderuccio F. The Causative H +/K+ ATPase antigen in the pathogenesis of autoimmune gastritis. Immunol Today. 2000;21:348-54.
9. Toh BH, Whittingham S, Alderuccio F. Gastritis and pernicious anemia. Autoimmune Dis. 2006;39:527-46.
10. Toh BH, Alderuccio F. pernicious anemia. Autoimmunity. 2004;37:357-61.
11. Samloff IM, Kleinman MS. Turner MD, et al. Blocking and binding antibodies to intrinsic factor and parietal cell antibody in pernicious anemia. Gastroenterology. 1968;55:575-84.
12. Davidson RJ, Atrah HI, Sewell HF, Longitudinal study of circulating gastric antibodies in pernicious anemia. J Clin Pathol. 1989;42:1092-5.
13. Lahner E, Norman GL, Severi C, et al. Reassessment of intrinsic factor and parietal cell autoantiboides in atrophic gastritis with respect to cobalamin deficiency. Am J Gastroenterol. 2009;104:2071-2079.
14. Birn H, Willnow TE, Nielsen R, et al. Megalin is essential for renal proximal tubule reabsorption and accumulation of transcobalamin-B(12). Am J Physiol Renal Physiol. 2002;282(3):F408-16.
15. Ronald Hoffman Edward Benz Leslie Silberstein Helen Heslop Jeffrey Weitz John Anastas. (2012). Megaloblastic Anemias. Hematology, basic principles and practice, Chapter 37 (6th edition); 473-504.
16. Ayesh MH, Jadalah K, Al Awadi E, et al. Association between Vitamin B12 and anti – parietal cells and anti – intrinsic factor antibodies among adult Jordanian patients with Helicobacter pylori infection. Braz J Infect Dis. 2013;17(6):629-32.
17. Loukili NH, Noel E, Blaison G, et al. Update of pernicious anemia. A retrospective study of 49 cases. La Revue de Medicine Interne. 2004;25(8):556-561.
18. Amarapurkar DN, Amarapurkar AD. Intrinsic factor antibody negative atrophic gastritis; is it different from pernicious anaemia? Trop Gastroenterol. 2010;31(4):266-70.
19. Desai HG, Anita FP. Vitamin B12 Malabsorption due to intrinsic factor deficiency in Indian subjects. Blood. 1972;40:747-53.
20. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. Am J Clin Nutr. 2011;94(1):348S-358S.
21. Remacha AF, Sardà MP, Canals C, et al. Role of serum holotranscobalamin (holoTC) in the diagnosis of patients with low serum cobalamin. Comparison with methylmalonic acid and homocysteine. Ann Hematol. 2014;93(4):565-9.
22. Remacha AF, Wright I, Fernández-Jiménez MC, et al. Vitamin B12 and folate levels increase during treatment of iron deficiency anaemia in young adult woman. Int J Lab Hematol. 2015;37(5):641-8.
23. Carmel R. Reassessment of the relative prevalence's of antibodies to gastric parietal cell and to intrinsic factor in patients with pernicious anemia: influence of patient age and race. Clin Exp Immunol. 1992;89(1):74-77.
24. Pedersen AB, Mosbeek J. Morbidity of pernicious anemia. Incidence, prevalence and treatment in Danish country. Acta Medica scandinavica. 2009;185:449-452.
25. Dali-Youcef N, Andrès E. An update on cobalamin deficiency in adults. QJM. 2009;102(1):17-28.
26. Wun Chan JC, Yu Liu HS, Sang Kho BC, et al. Study of 181 patients in a Hong Kong Hospital. Medicine (Baltimore). 2006;85:129-138.
27. Khan S, Del-Duca C, Fenton E, et al. Limited value of testing for intrinsic factor antibodies with negative gastric parietal cell antibodies in pernicious anaemia. J Clin Pathol. 2009;62(5):439-41.
28. Hershko C, Ronson A, Souroujon M, et al. Variable haematological presentation of autoimmune gastritis; age related progression from iron deficiency to cobalamin depletion. Blood. 2006;107(4):1673-9.
29. Feldt-Rasmussen U, Bech K, Bliddal H, et al. Auto antibodies, immune complexes and HLA- D in thyrogastic autoimmunity. Tissue antigens. 1983;22:342-347.
30. Desai HG, Dighe PK, Borkar AV. Parietal cell and intrinsic-factor antibodies in Indian subjects. Scand J Gastroenterol. 1968;3(3):321-6.
31. Dixon MJ. Helicobacter pylori and peptic ulceration: histopathological aspects. J Gastroenterol Hepatol. 1991;6:125-30.
32. Claeys D, Faller G, Appelmelk BJ, et al. The gastric H+, K+-ATPase is a major autoantigen in chronic Helicobacter pylori gastritis with body mucosa atrophy. Gastroenterology. 1998;115(2):340-7.
33. Adlekha S, Chadha T, Krishnan P, et al. Prevalence of helicobacter pylori infection among patients undergoing upper gastrointestinal endoscopy in a medical college hospital in kerala, India. Ann Med Health Sci Res. 2013;3(4):559-63.
34. Thirumurthi S, Graham DY. Helicobacter pylori infection in India from a western perspective. Indian J Med Res. 2012;136(4):549-62.
35. Choudhuri G, Mohindra S. Epidemiology of Helicobacter pylori in India. Indian J Gastroenterol. 2000;19 Suppl 1:S3-5.