Misdiagnosed vitamin B12 deficiency a challenge to be confronted by use of modern screening markers

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Abstract

Vitamin B12 deficiency is one of the common conditions in the elderly population leading to confusion, depression, memory loss and balance problems. Unfortunately there is no gold standard test for the diagnosis of cobalamin deficiency and a wide range of variation in reference levels according to country, and laboratory assay used. This poses a problem in diagnosing this condition making it a commonly misdiagnosed medical entity. There is current emphasis on need for clearer guidelines and much research is still being done to pave ways to determine better reference values for serum B12 and other screening tests. It is advised that screening methods are used adjunctively for the purpose of screening individuals.

Keywords: Vitamin B12, Europe, Metformin.

Introduction

Vitamin B12 also known as cobalamin, is an essential micronutrient required for proper functioning of the body cells. Vitamin B12 deficiency has been estimated to be more than 20 patient in the population of UK.[1] It is not necessarily limited to the elderly as it also occurs in vegans, after gastric bypass surgery, Diabetes mellitus type 2 and many other conditions and with drugs such as proton pump inhibitors and Metformin.[2] Therefore it is suggested that people at risk should be screened to prevent misdiagnosis. It takes part in two known reactions of body cells as a coenzyme and is involved with myelination of nerves ensuring conduction of nerve impulses and formation of red blood cells. vitamin B12 deficiency is a common condition associated with many other medical conditions such as crohns disease, diabetes mellitus, multiple sclerosis, Alzheimer's disease, dementia, autoimmune diseases as thyroditis, rheumatoid arthritis and Addison's disease. Pernicious anaemia which is considered to be an autoimmune disease itself is specially associated with autoimmune disorders.[3] Knowledge of important key points pointing to the diagnosis of this condition is essential for every clinician so we will try to highlight some significant diagnostic criteria in this review.

How common is it?

In the United Kingdom during the period 2008-2009[4] according to NHS statistics 1152 hospital admissions due to vitamin B12 deficiency resulted in finished consultations, whereas 375 were emergency cases another 873 required hospital admission resulting in a total of 3092 bed days and another 376 were put on a waiting list. Highest figures aetiology wise were for intrinsic factor deficiency and according to age in above 75 years (Table-1).

This shows vitamin B12 can be diagnosed in a primary care setting and introduction of screening in carefully targeted risk groups seems sensible. If detected earlier, prognosis may improve as randomised studies on

Table-1:

Primary diagnosis: 4 character code and description	Finished consultant episodes	Admissions	Male	Emergency	Waiting list	Age 0-14	Age 15-59	Age 60-74	Age 75+	Day case	FCE Bed Days
D51.0 Vitamin B12 deficiency anaemia due to intrinsic factor deficiency	643	487	204	211	140	2	174	152	315	214	1661
D51.1 selective vitamin B12 malabsorption with proteinurea	3	3	1	1	0	0	3	0	0	2	0
D51.3 Other dietary vitamin B12 deficiency anaemia	44	29	16	18	7	0	15	13	16	9	122
D51.8 Other vitamin B12 deficiency anaemia's	54	41	27	21	8	1	16	8	29	15	185
D51.9 Vitamin B12 defic anemia unspecified	208	313	179	124	121	3	136	81	188	181	1124

elderly had shown that daily intake of 1-2mg of cyanocobalamin resulted in normalisation of metabolic signs and improvement in neurological symptoms in terms of power, gait, memory, vibration sensations and parasthesia's[5] but in some trials it was argued that such long term oral supplements were not effective in reversal of haematological features such as macrocytosis, basal stippling etc.

In the UK as it happens in many other countries the fact remains that vitamin B12 deficiency is often misdiagnosed or under diagnosed. The most common conditions, it is confused with are multiple sclerosis, Alzheimer's disease, lateral amyotrophic sclerosis and peripheral neuropathies. In fact there are no figures to prove the misdiagnosis but the fact that so many older people are presenting with vitamin B12 deficiency suggest there is a tendency to under diagnose this condition.

Is diagnosing vitamin B12 a challenge?

The biggest reason for misdiagnosis is due to the strikingly similar neurological symptoms these conditions share with one another.

Now as vitamin b 12 stores may take 5 to 10 years to be depleted so clinicians may miss the diagnosis as serum B12 levels which is the primary diagnostic test may appear normal. One also has to bear in mind that the vitamin B12 levels can be low independent of Macrocytic anaemia.[6] Undoubtedly it is also costing the NHS a lot of money due to number of admissions preventable as well as costs that incur if complications develop. Vitamin B12 deficiency may result in complications such as sub combined degeneration of spinal cord. Moreover people having pernicious anaemia are at a very high risk of developing gastric carcinoma. Its speculted It costs only above £3.00 to carry out a basic investigation as compared to £22 for 6 injections and £280 for antidepressants.[7]

Considering all the facts above Vitamin B12 levels may be screened but as this could be costly making it possible only to screen risk groups who are either prone to develop severe neurological symptoms or are already developing them so follow ups should be encouraged in those who have mild symptoms.

Dementia is also linked to very low vitamin B12 levels. About 1.5 percent of UK population suffers from dementia that is about 820,000 people.[8] An interesting controlled trial study was carried out at the Oxford University in which one group was given vitamin B12 and other group received placebo. The results indicated on an average 30 percent less brain shrinkage was seen in those receiving vitamin B12 being 50 percent in those who started on highest dose of vitamin B12. This provides evidence that maybe vitamin B12 deficiency is preventable and linked to other neurological diseases and psychiatric disorders as well.[9]

Pregnant women who have vitamin B12 deficiency may give birth to vitamin B12 deficient babies. The incidence of neonatal vitamin B12 (cobalamin) deficiency because of maternal deficiency was determined by a research group surveying United States newborn screening programmes. Thirty-two vitamin B deficient babies were identified which is .88/100000.[10] Pregnant women should be screened for their risk of inadequate intake/malabsorption of vitamin B12.Vitamin B12 secondary to maternal vitamin B12 deficiency leads to slow neurological development, hypotonias and cerebral atrophy and seizures.[11] Children born with an inherited disorder of vitamin B(12) metabolism combined methylmalonic acidaemia and homocystinuria, cobalamin C (cblC) type, is caused by mutations in MMACHC in addition to these neurological symptoms will have renal and hepatic impairment as well.[12]

What are clinical symptoms?

There is a wide spectrum of clinical manifestations of B12 deficiency affecting nearly every system of the body which must be recognised before screening:

General symptoms like tiredness, malaise, breathlessness, fatigue, tinnitus and lethargy.

Cutaneous lesions are Pallor, yellow tinge to the skin, vitiligo, hyperpigmentation, angular stomatitis and hair changes.[13]

Oral symptoms are ulceration and beefy red tongue specially margins with swollen papillae.[14]

Neurological signs and symptoms such as pins and needles, numbness, loss of balance, falls, loss of pain and vibration sensations, memory loss, decreased power of limbs or peripheral neuropathy which is mostly symmetrical. Positive Babinskis sign and rarely signs of autonomic dysfunction such as urine incontinence and impotence may be encountered.

Gastrointestinal symptoms such as diarrhoea, abdominal cramps. Psychiatric symptoms such as depression, personality change, mania, hallucinations delusions, irritability, mood changes may develop.

Cardiovascular symptoms and signs such as murmurs, heart failure and syncope are also seen.

Complications are Peripheral neuropathy's specially symmetrical mostly involving lower limb but can ascend to upper limbs as well, Other neuropathy's are encephalopathy's, myeloencephlopathy's, myelopathy's. Optic neuropathy's, though uncommon, are visual evoked potentials can be abnormal in even up to 77 percent of

patients. Other more common complication' are Macrocytic anaemia and sub acute combined degeneration of spinal cord. On MRI Scans white patchy areas are seen showing patchy involvement of spinal cord.[3]

MRI scan can also have its own significance as an incidental finding Vitamin B12 status may be investigated for and early recognition leads to early intervention and initiation of treatment, which may improve symptoms.MRI Cranial findings are brain atrophy with signs of retarded myelination frontal and temporal lobes being most affected.[15] In subacute combined degeneration of spinal cord MRI of cervical spine shows hyper intensity of areas C2-C5 with typical symmetrical T2 high Signal changes affecting mostly posterior column, sometimes lateral column due to expansion of the spinal cord. Sometimes these are known as long tape lesions However an unusual case of anterior as well as posterior column involvement on cervical MRI Scan has been reported.[16] Rarely pyramidal, spinocerebellar tracts and cerebellum are also affected.[17] MRI scan has its own diagnostic value when symmetrical neurological findings are present. Nerve conduction studies show abnormal peroneal and sural nerve conduction and Tibial somatosensory evoked potential may show abnormality. Visual evoked potentials have been found to be abnormal[18] but mostly don't clinically become apparent showing that B12 tissue damage manifests at later stages of vitamin B12 deficiency.

What tests are available?

Unfortunately only primary tests are widely available such as routinely performed complete blood picture and serum B12 levels. Blood smear and reticulocyte count are also included in primary screening evaluation. The tests lack sensitivity as the B12 levels depletion as well as Macrocytic anaemic picture may take years to develop despite the risk being there .Also the cut off levels for different laboratories are different. Studies have shown that the higher the cut off values used such as higher than 200 picograms/ml, the more percentage of people having this deficiency are actually diagnosed. The serum B12 levels are generally divided into 3 categories:[19]

Likely vitamin B12 deficiency	<148picomole/L(200 Pico grams/ml)
Possible vitamin B12 deficiency	148-258 picomoles/L (201-350 Pico grams/ml)
Unlikely vitamin B12 deficiency	>258 picomoles/L(350 Pico grams/ml)

Whenever the gray zone of 148-258 picomole/L is present, other specific tests of MMA (methyl malonic acid) or holo tc should be considered for purpose of secondary screening.

Serum B12 deficiency can occur without Macrocytic anaemia and blood changes as patients with neurological deficits due to vitamin B12 deficiency have absent haemotogical features.

If there are blood changes B12 Haemoglobin may or may not be decreased, Mcv may or may not be raised and if raised, it indicates further evaluation of vitamin B12 deficiency. It may rise even before fall in haemoglobin levels but its absence does not rule out B12 deficiency and it is not a specific marker as using it solely would miss 84 percent cases. Its normal range is 80-96 fl.[20] MCV can be

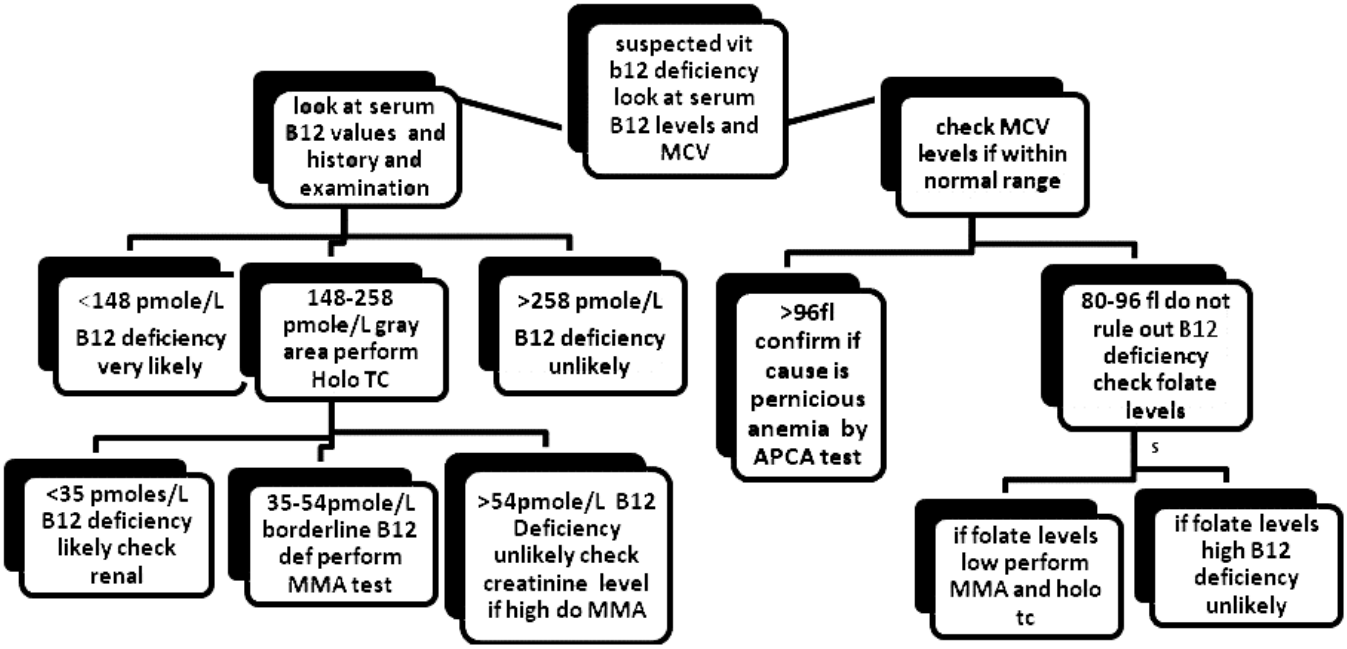

Figure-1: Suggested approach of investigating vitamin B12 deficiency:

within normal range despite B12 deficiency specially with concomitant iron deficiency, drugs like Hydroxyurea for example and there may be normocytic anaemia or even microcytic anaemia in some cases:

80-100 fl: 25 percent probability of successful diagnosis

115-129 fl: 50 percent probability of successful diagnosis

Above 130 fl: 100 percent probability.[19]

LDH Levels may be raised and there may be hyperchlorhydria.

On Blood smear following changes indicate pernicious anaemia:

Anisocytosis, basal stippling, poikilocytosis, abnormal platelets, hyper-segmented neutrophils, thrombocytopenia and presence of Howell jolly bodies. Other causes of Macrocytic anaemia are alcohol abuse, certain inherited disorders, drugs that affect DNA, such as chemotherapy drugs, leukaemia, Myelodysplastic syndrome, and myelofibrosis.[21]

Discussion

There are various tests that maybe used for purpose of screening such as serum B12, holo tc, homocystein, serum or urine Methylmalonic acid (MMA) but out of them none is a gold standard and all have certain advantages, and disadvantages. Some have disputed and varied reference range and are not very specific. Some are highly specific but coexistent medical conditions can limit their use.

Serum B12 is the primary test routinely used which can give inaccurate results in high folate levels, and vitamin C intake.[22] In cases of low Folate levels, Oral contraceptives, pregnancy, multiple myeloma, transcobalamin 1 deficiency, Aplastic anaemia, hairy cell leukaemia, Waldenstorms macroglobinaemia and despite showing vitamin B12 deficiency may be presumed not to be B12 deficient. Conversely false negatives showing normal or high B12 levels despite coexistent cobalamin deficiency are in cases of recent cobalamin injections ,abnormalities of transcobalamin transport, metabolism or increased levels of transcobalamin, circulating antitranscobalamin antibody, leukaemia's and myeloproliferative disorders, hypereosinophilic syndrome and leukemoid reaction, metastatic cancer and hepatocellular cancer, liver or renal failure.[23] Inherited conditions like Spastic paraplegia and beta thalassemia have been found to mask vitamin B12 deficiency.[24]

MMA or Methylmalonic acid is a more specific test being suggested as a gold standard but very seldom performed though is widely available but usually reserved for borderline cases, deteriorating neurological status or for follow-up. B12 catalyzes conversion of methylmalonyl-CoA to succinyl-CoA. Absence of B12 causes accumulation of methylmalonyl-CoA and subsequent conversion to Methylmalonic acid (MMA), resulting in elevated serum MMA levels in B12 deficiency states.[25] Specificity is 98 percent and can be false positive in case of deteriorating renal status. Its use hasn't been much promoted despite its wide use in research trials. Sometimes this fact may result in missing vitamin B12 deficiency if a more specific test such as the MMA is never done. Furthermore this may in fact worsen prognosis and quality of life in such cases. So can secondary screening be done where suspected by GPs and neurologists? The best test would be urinary MMA Levels which are elevated when vitamin B12 is low. Possibly neurologists could screen vitamin B12 deficiency but the problem is MMA is not a cost effective way of screening and yet no agreed consensus exists on a single screening method.

Another test is the active B12, more commonly known as holo transcobalamin or the holoTC test.

This test though not as specific as the urinary methyl malonic acid or MMA Test but is more superior to the serum B12 assay. Serum B12 assay though widely used has the disadvantage that it does not differentiate between the active B12, which constitutes roughly 20 percent of B12 bound to Transcobalamin 2 and the inactive form which is 80 percent. Serum B12 detects all types of vitamin B12 giving inaccurate results. It is estimated that this method used alone may result in up to 50 percent misidagnosis[26] whereas the holo tc test is more specific only picking up the active B12 which is subjected to tissue intake making it a more valuable test. So it is apparent that both tests should be used in conjunction for screening. It has been suggested that where serum B12 levels fall in the gray zone of 148-258 picogram/L the secondary screening method must be applied such as holo TC or serum/urinary MMA, which are more modern markers. Advantages of this test besides being an early B12 marker, are that it is a comparatively better marker of long term status and does not show great circadian variation. It can be done in non-fasting subjects .Limitation of holo tc test is that it has been observed in studies that it does not pick up vitamin B12 deficiency in malabsorption of vitamin B12 such as pernicious anaemia as absorption status seems to have an effect on transcobalamin 2.[27] Therefore parietal cell antibodies test still has its role in diagnosing pernicious anaemia.

If all tests are compared to one another persons with normal level of vitamin B12 may have raised MMA (>271 nmol/L) and lowered concentrations of holoTC (<35 pmol/L), owing to intracellular, metabolically manifest (functional) vitamin B12 deficiency.[25] By contrast, lowered concentrations of B12 and normal MMA indicate a false

positive finding and should be re-investigated. Holo tc levels are decreased when B12 levels decrease signifying a direct relationship between holo tc and B12. Holo tc levels below 35pmole/litre are considered subnormal.[28] Levels in the range of 35-54 pmole/litre are considered borderline low.[29] Any levels higher than this indicate that vitamin B12 deficiency is unlikely unless there is renal insufficiency determined by creatinine levels (Table-2).

Table-2:

Methylmalonic acid reference values	
Normal serum MMA	0.07-0.27micromole/litre[30]
Laboratory cut off	0.396micromole/litre[31]
Urinary MMA	0.58-3.56micromole/mmole/litre Cr[32]

Homocystein levels are also raised in vitamin B12 deficiency. Methyltetrahydrofolate and vitamin B12 (cobalamin) are required for remethylation of homocystein (Hcy) to methionine, which is mediated by methionine synthase and methionine synthase reductase.[33] Therefore, intracellular availability of vitamin B12 influences total Hcy (tHcy) plasma concentrations. It is considered a risk factor for cardiovascular disease.

Homocystein levels are also a better indicator of vitamin B12 deficiency as compared to serum B12 values as blood index Mcv has been found to have a direct relationship with Homocystein levels. Normal Homocystein levels range from 2.2-13.2 micromoles/litre.[34] Its only disadvantage is that there is no specific test for it and unlike MMA is elevated in many other conditions.[35]

Screening for Pernicious Anaemia:

Pernicious anaemia is an autoimmune disease as well as end stage of Atrophic body gastritis that causes acquired vitamin B12 deficiency. It is associated with many other autoimmune diseases such as diabetes and autoimmune thyroditis. There are two types of intrinsic auto antibodies type 1 called as blocking and type 2 called as precipitant antibodies.[36] In addition to intrinsic factor and antiparietel antibodies tests there are also serological markers such as gastrin stimulation test and pepsinogen test {lowered levels).[37] With the Schillings test now becoming obsolete newer tests such as gastric IF output under 200 U/h post pentagastrin stimulation (N > 2000 U/h) is specific for PA rather than recently advocated raised serum gastrin levels and antibodies used as diagnostic criteria which are rather non-specific.[38] Once vitamin B12 deficiency is detected on treatment with parentral vitamin B12 injections does not result in any improvement of symptoms or haematological results then pernicious anaemia must be suspected. In addition to vitamin B12 levels and complete blood picture, auto antibodies against the gastric parietal cells antigen H+/K+ ATPase and intrinsic factor,[39] endoscopy for signs of gastritis maybe performed to confirm the diagnosis .A suggested approach is shown in Figure-2.

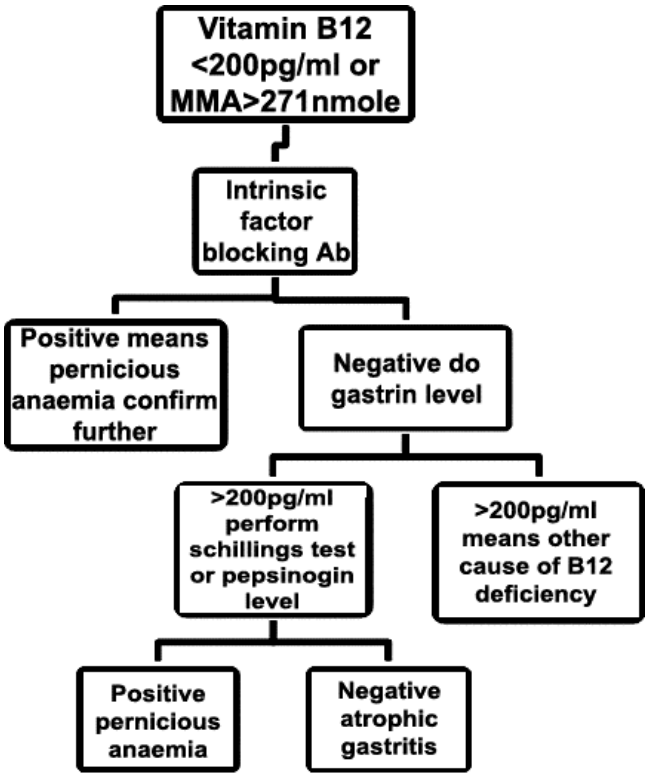

Figure-2: Reference from Current Concepts in the Diagnosis of Cobalamin Deficiency, Neurology 1995; 45: 1435-1440).

Approach to diagnosis of pernicious anaemia:

Other aspects of screening are screening of individuals with inherited disorders. It has been hypothesized that changes in holo-TC in response to a supplemental dose of vitamin B-12 may be used to assess vitamin B-12 absorption. Imurseland-Grasbeck syndrome is a rare inherited autosomal disorder characterised by vitamin B12 deficiency with selective absorption of B12 and proteinurea is another cause of malabsorption. It results in recurrent infections and failure to thrive mostly appearing at childhood and results in megaloblastic anaemia as well as neurological symptoms which can be screened for in childhood.

Screening tests must also be used as follow up tools such as in gastric bypass surgery. It is easier and more efficient to treat vitamin B12 deficiency as other nutritional deficiencies in the preoperative stage and can be prevented.

Therefore, preoperative detection and correction are crucial. In the postoperative period, blood tests should be conducted every 3 months in the first year after operation, every six months in the second year and annually thereafter.[39]

Key Points:

If either history or examination even apparently minor symptoms such as tingling, pins and needles always rule out vitamin B12 Deficiency first. Referral to a neurologist may be indicated in such cases where severe neurological deficit is present.

In cases of memory loss or neurological symptoms of vitamin B12 deficiency should be ruled out.

Most cases of multiple sclerosis and dementia, atrophic gastritis, Crohn's disease, Coeliac disease ,intestinal or gastric resection, certain drugs such as proton pump inhibitors,H2receptor blockers, Metformin especially long-term will mostly have poor vitamin B12 status and treatment with vitamin B12 may benefit these patients.

Never forget to ask about dietary habits and intake of folate, vitamin c and supplements as if serum B12 is low and urine MMA or shillings test as appropriate may be considered later.

Current Research Questions:

Vitamin B12 deficiency screening before and after gastric bypass using urinary MMA?

Pregnant women especially vegan screened for vitamin B12 deficiency and how useful B12 therapy can be in these women?

If a screening programme was introduced to detect individuals suffering from dementia in care homes what outcomes would be expected?

Conflict of interest and funding declaration:

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References

1. Andre's E, Loukili NH, Noe E, Kaltenbach G, Abdelgheni MB, Perrin AE, et al. Vitamin B12 (cobalamin) deficiency in elderly patients. CMAJ 2004; 171: 257-9.
2. Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM. Risk factors of vitamin B (12) deficiency in patients receiving metformin. Arch Intern Med 2006; 166: 1975-9.
3. Misra UK, Kalita J. Comparison of clinical and electro diagnostic features of vitamin b12 deficiency neurological syndromes with and without antiparetal bodies. Postgrad Med J 2007; 96: 124-7.
4. Primary diagnosis 2008-09 The Health and Social Care Information Centre. (Online) 2011 (Cited 2011 Feb15). Available from URL: http://www.ic.nhs.uk/webfiles/rublications/AandE/AandE809/AE_attendences_in_England%20_expermental_statistics_%202008_09_vs.pdf. Nhs stastics 2008-09.
5. Butler CC, Vidal-Alaball J, Cannings-John R, McCaddon A, Hood K, Goringe A, Mcdowell I, et al. Oral vitamin B12 versus intramuscular vitamin B12 for vitamin B12 deficiency: a systematic review of randomized controlled trials. Fam Pract 2006; 23: 279-85.
6. Lindenbaum J, Healton EB, Savage DG, Brust JC, Garrett TJ, Podell ER, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. Engl J Med 1988; 318: 1720-8.
7. Vitamin B12 research. (Online). 2002-2011 (Cited 2001 Feb16). Available from URL: http://www.msrc.co.uk/.
8. Martin Knapp, Martin prince. Dementia UK. London: Alzheimer's society; 2007, pp 189.
9. Smith AD, Smith SM, de Jager CA, Whitebread P, Johanston C, Agacinski G, et al. Homocysteine lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trail. PLoS One 2010; 5: e12244.
10. Hinton CF, Ojodu JA, Fernhoff PM, Rasmussen SA, Scanlon KS, Hannon WH.Maternal and neonatal vitamin B12 deficiency detected through expanded newborn screening--United States, 2003-2007. J Pediatr 2010; 157: 162-3.
11. Incecik F, Hergüner MO, Altunba?ak S, Leblebisatan G. Neurologic findings of nutritional vitamin B12 deficiency in children. Turk J Pediatr 2010; 52: 17-21.
12. Weisfeld-Adams JD, Morrissey MA, Kirmse BM, Salveson BR, Wasserstein MP, McGuire PJ, et al. Newborn screening and early biochemical follow-up in combined methylmalonic aciduria and homocystinuria, cblC type, and utility of methionine as a secondary screening analyte. Mol Genet Metab 2010; 99: 116-23.
13. Kannan R, Ng MJ. Cutaneous lesions and vitamin B12 deficiency: an often-forgotten link. Can Fam Physician 2008; 54: 529-32.
14. Lehman JS, Bruce AJ, Rogers RS. Atrophic glossitis from vitamin B12 deficiency: a case misdiagnosed as burning mouth disorder. J Periodontol 2006; 77: 2090-2.
15. Lövblad K, Ramelli G, Remonda L, Nirkko AC, Ozdoba C, Schroth G. Retardation of myelination due to dietary vitamin B12 deficiency: cranial MRI findings. Pediatr Radiol 1997; 27: 155-8.
16. Karantanas AH, Markonis A, Bisbiyiannis G. Subacute combined degeneration of the spinal cord with involvement of the anterior columns: a new MRI finding. Neuroradiology 2000; 42: 115-7.
17. Katsaros VK, Glocker FX, Hemmer B, Schumacher M. MRI of spinal cord and brain lesions in subacute combined degeneration. Neuroradiology 1998; 40: 716-9.
18. Misra UK, Kalita J, Das A. Vitamin B12 deficiency neurological syndromes: a clinical, MRI and electrodiagnostic study. Electromyogr Clin Neurophysiol 2003; 43: 57-64.
19. Snow CF. Laboratory diagnosis of vitamin b12 and folate deficiency ;guide to a primary care physician Arch intern med 1999; 159: 1289-98.
20. Oosterhuis WP, Niessen RW, Bossuyt PM, Sanders GT, Sturk A. Diagnostic value of the mean corpuscular volume in the detection of vitamin B12 deficiency. Scand J Clin Lab Invest 2000; 60: 9-18.
21. Kaferle J, Strzoda CE. Evaluation of macrocytosis. Am Fam Physician 2009; 79: 203-8.
22. Selhub J, Morris MS, Jacques PF. In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. Proc Natl Acad Sci USA 2007; 104: 19995-20000.
23, Ralph C, David SR. Disorders of Cobalamin and Folate Metabolism. In: Robert I.H, Samuel E.L editors Thomas PS. Blood: principles and practice of hematology. 2nd ed. Philadelphia: Lippincott Williams and Wilkins; 2003, pp 1361-97.
24. Bilic E, Bilic E, Zagar M, Juric S. Masked deficit of vitamin B12 in the patient with heterozygous beta-thalassemia and spastic paraphrases. Acta Neurol Belg 2004; 104: 173-5.
25. Herrmann W, Obeid R. Causes and early diagnosis of vitamin B12 deficiency. Dtsch Arztebl Int 2008; 105: 680-5.
26. Oh R, Brown DL. Vitamin B12 deficiency Am Fam Physician 2003 ; 67: 979-86.
27. Chen X, Remacha AF, Sardar MP, Carmel R. Influence of cobalamin deficiency compared with that of cobalamin absorption on serum holo-transcobalamin II. Am J Clin Nutri 2005; 81: 110-4.
28. Bhat DS, Thuse NY, Lubree HG, Joglekar CV, Naik SS, Ramadas LV, et al. Increases in Plasma Holotranscobalamin Can Be Used to Assess Vitamin B-12 Absorption in Individuals with Low Plasma Vitamin B-12. J Nutr 2009; 139: 2119-23.
29. Loikas S, Koskinen P, Irjala K, Löppönen M, Isoaho R, Kivelä SL, et al. Renal impairment compromises the use of total homocysteine and methylmalonic

acid but not total vitamin B12 and holotranscobalamin in screening for vitamin B12 deficiency in the aged. Clin Chem Lab Med 2007; 45: 197-201.

30. Kuzminski AM, Del Giacco EJ, Allen RH, Stabler SP, Lindenbaum J. Effective treatment of cobalamin deficiency with oral cobalamin. Blood 1998; 92: 1191-8.
31. Holleland G, Schneede J, Ueland PM, Lund PK, Refsum H, Sandberg S. Cobalamin deficiency in general practice. Assessment of the diagnostic utility and cost-benefit analysis of methylmalonic acid determination in relation to current diagnostic strategies. Clin Chem 1999; 45: 189-98.
32. Rasmussen K. Solid phase sample extraction for rapid determination of methylmalonic acid in serum and urine by a stable isotope dilution method. Clin Chem 1989; 35: 260-4.
33. Loehrer FM, Schwab R, Angst CP, Haefeli WE, Fowler B. Influence of oral S-adenosylmethionine on plasma 5-methyltetrahydrofolate, S.adenosylhomocysteines, homocysteine and methionine in healthy humans. J Pharmacol Exp Ther 1997; 282: 845-50.
34. Haltmayer M, Mueller T, Poelz W. Erythrocyte mean cellular volume and its relation to serum homocysteine, vitamin B12 and folate. Acta Med Austriaca 2002; 29: 57-60.
35. Zittoun J, Debril J, Jarret J, Sultan C, Zittoun R. [Search for anti-intrinsic factor antibodies in the diagnosis of Biermer's anemia]. Sem Hop 1975; 51: 229-32.
36. Lahner E, Annibale B. Pernicious anemia: new insights from a gastroenterological point of view. World J Gastroenterol 2009; 15: 5121-8.
37. Cattan D. Pernicious anemia: What are the actual diagnosis criteria? World J Gastroenterol 2011; 17: 543-4.
38. Sedláková L, Dubská L, Pr?cha M. [Pernicious anaemia--diagnostic benefit of the detection of autoantibodies against intrinsic factor and gastric parietal cells antigen H+/K+ ATPase]. Epidemiol Mikrobiol Imunol 2010; 59: 126-32.
39. Schweiger C, Keidar A. [Nutritional deficiencies in bariatric surgery patients: prevention, diagnosis and treatment]. Harefuah 2010; 149: 715-20, 748.