CASE REPORT

Reversal of isolated 20q deletion with vitamin B_{12} replacement in a patient with pernicious anaemia

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SUMMARY

Severe vitamin B_{12} deficiency is well known to cause morphological alterations in bone marrow. In rare instances, these myelodysplastic and megaloblastic changes can coexist with cytogenetic abnormalities. Here, we report a case of a 38-year-old African-American woman with pernicious anaemia, who was found to have an isolated 20q deletion and which resolved after vitamin B_{12} replacement. We also discuss various mechanisms in which vitamin B_{12} deficiency can lead to chromosomal abnormalities. A literature review is also performed to evaluate various other chromosomal aberrations associated with B_{12} deficiency.

BACKGROUND

Folic acid and vitamin B_{12} (B_{12}) play an important role in DNA synthesis and gene expression. Although there are numerous in vivo and in vitro studies linking folate to chromosomal abnormalities and mutagenesis, the data on B_{12} is however surprisingly limited to few case studies. To the best of our knowledge, this is the first reported case of an isolated 20q deletion secondary to vitamin B12 deficiency. Our report adds new findings to the current existing literature of various other cytogenetic abnormalities seen in the bone marrow from severe B_{12} deficiency. This manuscript also highlights the importance to exclude B_{12} deficiency in patients with new diagnosis of myelodysplasia with cytogenetic abnormalities.

CASE PRESENTATION

A 38-year-old African-American woman was initially referred to our haematology clinic for evaluation of macrocytic anaemia. The patient was in a normal state of health until 6 months prior to this presentation. She then experienced progressively worsening fatigue, heart burn, nocturnal cough and weight loss over 20 lbs. Pertinent physical examination and vital signs were grossly unremarkable. Initial laboratory findings were as follows: haemoglobin (Hb): 10 g/dL, mean corpuscular volume: 110 fL, white cell count (WCC): 3.92×10^3/µL, platelets: 281 000/µL, reticulocyte count: 1.4%, serum B_{12}: 4187 pg/mL, serum folate: 33 ng/mL, iron: 84 µg/dL, total iron binding capacity: 251 µg/dL, ferritin: 119 ng/mL, transferrin saturation: 33%, lactate dehydrogenase: 205 U/L, haptoglobin: 32 mg/dL, thyroid stimulating hormone: 0.650 µIU/mL, copper: 166 µg/dL. Hepatitis and Human Immunodeficiency Virus 1 and 2 serology was negative. Peripheral blood smear revealed macrocytosis, anisocytosis, tear drop cells, adequate platelets and no evidence of dysplastic cells. Secondary to peripheral blood examination revealing macrocytic red blood cells, tear drop cells in the context of normal B_{12} levels, a bone marrow biopsy was suggested, which was declined by the patient. Six months later after being lost to follow-up, she presented to the emergency room with bilateral leg weakness and her physical examination revealed marked skin hyperpigmentation, glossitis, moderately increased muscular tone, decreased sensation to touch and pressure in the lower extremities, decreased reflexes in the ankle and knee joints and wide based gait.

INVESTIGATIONS

Repeat laboratory findings were: Hb: 8.6 g/dL, WCC: 2.06×10^3/µL, platelets: 193 000/µL, folate: 19.9 ng/mL. Bone marrow biopsy revealed hypercellular bone marrow with megaloblastic and dysplastic changes, 6% CD34+ blast cells and rare ringed sideroblasts. Metaphase cytogenetic studies on bone marrow aspirate showed del 20 q (figure 1). B_{12} this time was noted to be at 83 pg/mL, serum homocysteine: 218 µmol/L, serum methyl malonic acid: 23246 nmol/L. Antiparietal antibody and anti-intrinsic factor antibody were strongly positive. The initial finding of a falsely elevated B_{12} levels can be perceived as an erroneous laboratory finding.

DIFFERENTIAL DIAGNOSIS

At patient's initial presentation, as all the work up for macrocytic anaemia was negative, myelodysplasia was considered in the differential diagnosis. But, the differential diagnosis was soon narrowed to B_{12} deficiency when the patient presented later with severe ataxia along with classic neurological manifestations in the presence of macrocytic anaemia. The primary diagnosis of pernicious anemia was confirmed when the serology was positive for both anti-parietal and anti-intrinsic factor antibodies.

TREATMENT

The patient was subsequently treated with intramuscular B_{12} supplementation (1000 µg every day for 1 week, every 1 week for 4 weeks and monthly thereafter) with rapid resolution of all haematological parameters in a week and more gradual improvement of neurological symptoms.

OUTCOME AND FOLLOW-UP

Seventy days after B_{12} replacement, a repeat bone marrow biopsy was performed and revealed a normocellular bone marrow with no evidence of myelodysplastic features and 3% CD34+ blast cells. Repeat cytogenetic studies on bone marrow aspirate

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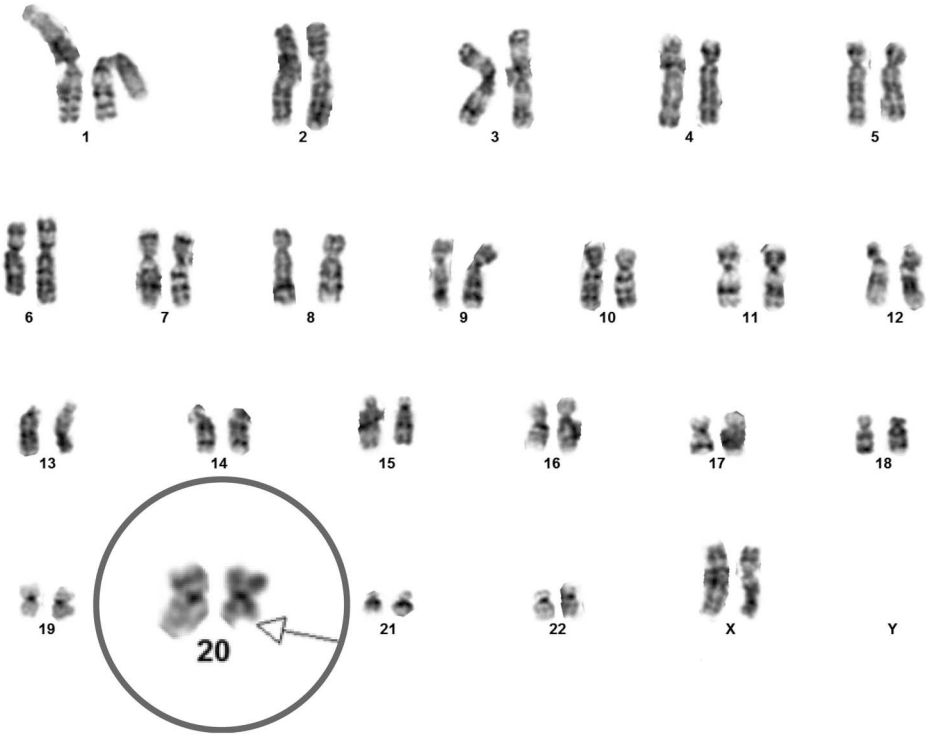

Figure 1 This figure demonstrates cytogenetic analysis revealing 20 q deletion before vitamin B_{12} replacement (chromosome 20 magnified (not to scale) to demonstrate the abnormality).

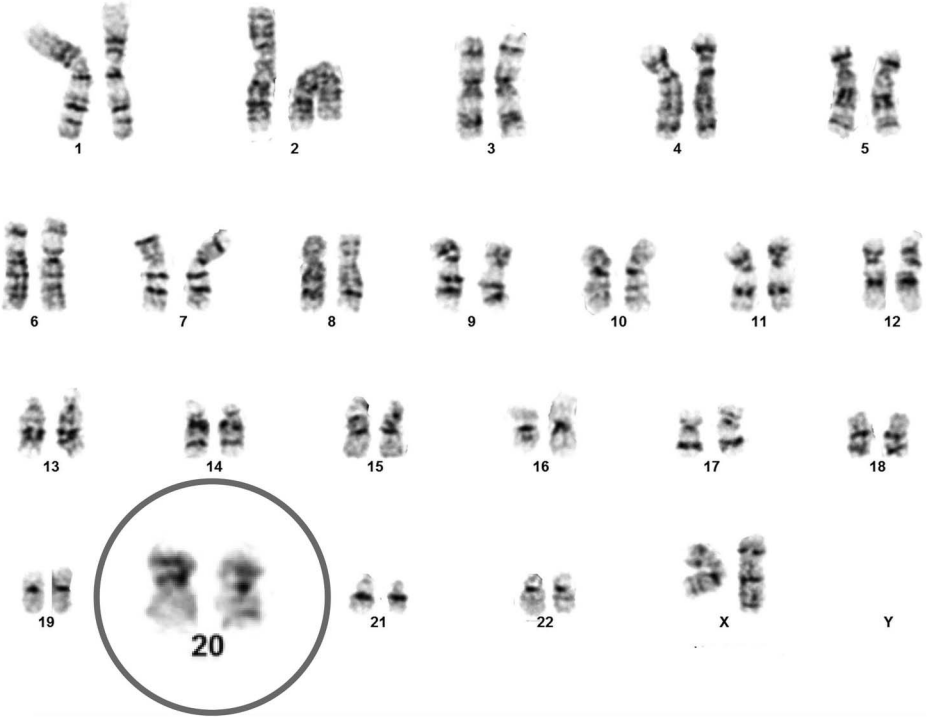

Figure 2 This figure demonstrates normal female karyotype and the reversal of the previously seen 20 q deletion after vitamin B_{12} replacement.

demonstrated normal female karyotype and reversal of prior noted del 20q (figure 2).

DISCUSSION

Dietary B_{12} (cobalamin) is absorbed in the distal ileum after binding to intrinsic factor secreted by the gastric parietal cells.[1] Cobalamin aids in the conversion of 5-methyl tetrahydrofolate (THF) to N 5,10 methylene THF, which is necessary for deoxy-thymidine monophosphate (dTMP) synthesis.[2] Cobalamin also mediates the conversion of homocysteine to methionine which is a precursor for S-adenosyl methionine, a methyl donor required for the maintenance of methylation patterns in DNA and the synthesis of formyl-THF.[2] Deficiency of B_{12} also leads to accumulation of homocysteine. B_{12} deficiency ultimately leads to trapping of folate, increased deoxy-uridine monophosphate (dUMP), which prevents incorporation of dTMP into the DNA. High intracellular ratio of dUMP/dTMP and subsequent increase in the deoxy-uridine triphosphate/deoxy-thymidine triphosphate (dUTP/dTTP) ratio results in misincorporation of uracil into the DNA strand.[3–5] Uracil DNA glycolase/apyrimidinic nuclease

Table 1 Other reports of B_{12} deficiency associated with chromosomal abnormalities

	Reports	N*	Deficiency	Comments
1	Ford, 1959[16]	1	B_{12}	Hypodiploidy; subsequently reported as an error
3	Astaldi *et al*, 1962[18]	1	B_{12}	Aneuploidy and changes in chromosome morphology Less pronounced after B_{12} replacement
4	Forteza and Baguena, 1963[28]	1	B_{12}	Aneuploidy
5	Kiossoglou *et al*, 1965[19]	3	B_{12}	Aneuploidy, chromosomal and chromatid breaks Changes persisted in one after B_{12} replacement
6	Jensen *et al*, 1967[22]	4	B_{12}	Increased chromosomal breaks
7	Heath, 1966[21]	14	B_{12} and folate	Chromosome breakage along with severe megaloblastic changes
8	Bottura *et al*, 1968[24]	1	B_{12}	Chromosomal breaks in metaphase; corrected after B_{12} replacement
9	Keller *et al*, 1970[20]	3	B_{12}	Chromosomal breaks resolved after B_{12} replacement in one patient
10	Lawler *et al*, 1971[23]	15	B_{12} and folate	Chromosomal breaks. Reversal seen in 48 hours with B_{12} replacement
11	Goh, 1981[29]	1	B_{12}	Del (18p), persisted with replacement. Unlikely from B_{12} deficiency
12	Wollman *et al*, 1996[26]	1	B_{12} and folate	Del (7q) with reversal after B_{12} replacement
13	Chintagumpala *et al*, 1996[25]	3	B_{12}	Spontaneous chromosomal fragility. Reversal with B_{12} replacement in one patient
14	Parmentier *et al* 2012[27]	1	B_{12}	Del (3p), with reversal of cytogenetic abnormalities after B_{12} replacement

*N, number of patients described in the case report.
B_{12}, vitamin B_{12}.

which corrects this mis-incorporation is overwhelmed in the continued absence of B12, and this lack of corrective process leads to accumulation in chromosomal fragile sites, chromosomal breaks and chromosomal deletions without any repair[6–7] and chromosomal deletions without any repair. Altered methylation of DNA is also hypothesised to play a role in mutagenesis and carcinogenesis.[8–10] Other plausible mechanisms for mutagenesis which have been studied in vitro include increased homocysteine levels,[11] and decreased telomere length[12] seen in patients with a B_{12} deficiency that affects genomic integrity. B_{12} deficiency can also cause micronucleus formation,[13–14] and accelerated apoptosis;[15] however, their role in mutagenesis is unknown.

Studies attempting to characterise chromosomal changes associated with B_{12} deficiency date back to as early as 1950s. But, a consistent demonstration of this finding was a challenge secondary to the limitations of laboratory techniques involved.[16] As the molecular techniques improved, various groups demonstrated multiple chromosomal abnormalities: increase in nuclear size,[17] hypodiploidy[18 19] and chromosomal breaks.[20–23] Some of these reports also demonstrate reversal of these abnormalities on replacement of B_{12}.[20 21 24 25] For example, B_{12} deficient patients with isolated 7q and 3p chromosomal deletions had reversal of these chromosomal findings on replacement therapy.[26 27] Table 1 summarises the various reports of chromosomal abnormalities which were associated with B_{12} deficiency.

Our case involved an isolated 20q deletion, a non-specific, yet a recurrent chromosomal abnormality seen in myelodysplastic syndrome (MDS).[30] Del (20q) preferentially involves erythroid and megakaryocytic precursors and this isolated abnormality when associated with MDS portends a favourable prognosis in terms of its overt progression into acute myeloid leukemia (AML).[31 32] It is to be noted that other conflicting reports about poor prognosis of del (20q) and higher conversion to AML have also been reported.[33] Del 20q is also known to be associated with myeloproliferative disorders, acute myeloid leukaemia, pure red blood cell aplasia and angioimmunoblastic lymphadenopathy with dysproteinaemia.[34] Morphologically bone marrow examination in a B_{12}-deficient individual can mimic those with frank myelodysplasia/leukaemia.[35–37] Cases have been reported where B_{12} deficiency and MDS/AML coexist.[38–39] Das *et al*[40] demonstrated that reversal of chromosomal breaks and despiralisation can take up to 12 weeks to normalise after B_{12} and folate supplementation.

Learning points

- This case demonstrates reversal of haematological parameters and cytogenetic abnormality after vitamin B12 replacement in a patient with pernicious anaemia.
- Reversible clonal cytogenetic abnormalities and bone marrow changes mimicking myelodysplastic syndrome may be seen in patients with severe B_{12} deficiency. It is prudent to identify and treat the B_{12} deficiency before making a misdiagnosis of myelodysplastic syndrome.
- Although B_{12} deficiency can cause chromosomal and hematopoetic abnormalities, its role in leukemogenesis is not clear. Further studies are needed to confirm if B_{12} deficiency can lead to mutagenesis and carcinogenesis.

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REFERENCES

1 Festen HP. Intrinsic factor secretion and cobalamin absorption: physiology and pathophysiology in the gastrointestinal tract. *Scand J Gastroenterol* 1991;26(Suppl 188):1–7.
2 Solomon LR. Disorders of cobalamin (vitamin B12) metabolism: emerging concepts in pathophysiology, diagnosis and treatment. *Blood Rev* 2007;21:113–30.
3 Blount BC, Ames BN. 2 DNA damage in folate deficiency. *Baillières Clin Haematol* 1995;8:461–78.
4 Blount BC, Mack MM, Wehr CM, *et al*. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci USA* 1997;94:3290–5.
5 Wickramasinghe SN, Fida S. Bone marrow cells from vitamin B12-and folate-deficient patients misincorporate uracil into DNA. *Blood* 1994;83: 1656–61.
6 Dianov GL, Timchenko TV, Sinitsina OI, *et al*. Repair of uracil residues closely spaced on the opposite strands of plasmid DNA results in double-strand break and deletion formation. *Mol Gen Genet* 1991;225:448–52.

7 Reidy JA, Zhou X, Chen AT. Folic acid and chromosome breakage I. Implications for genotoxicity studies. *Mutat Res Lett* 1983;122:217–21.
8 Wainfan E, Poirier LA. Methyl groups in carcinogenesis: effects on DNA methylation and gene expression. *Cancer Res* 1992;52(7 Suppl):2071s–7s.
9 Chen RZ, Pettersson U, Beard C, *et al*. DNA hypomethylation leads to elevated mutation rates. *Nature* 1998;395:89–93.
10 Ehrlich M. DNA methylation in cancer: too much, but also too little. *Oncogene* 2002;21:5400–13.
11 Huang RF, Huang SM, Lin BS, *et al*. Homocysteine thiolactone induces apoptotic DNA damage mediated by increased intracellular hydrogen peroxide and caspase 3 activation in HL-60 cells. *Life Sci* 2001;68:2799–811.
12 Pusceddu I, Herrmann M, Kirsch SH, *et al*. One-carbon metabolites and telomere length in a prospective and randomized study of B-and/or D-vitamin supplementation. *Eur J Nutr* 2016:1–2. DOI: 10.1007/s00394-016-1231-z
13 Jensen MK. Cytogenetic findings in pernicious anaemia. Comparison between results obtained with chromosome studies and the micronucleus test. *Mutat Res* 1977;45:249–52.
14 Fenech M. Micronucleus frequency in human lymphocytes is related to plasma vitamin B12 and homocysteine. *Mutat Res* 1999;428:299–304.
15 Koury MJ, Price JO, Hicks GG. Apoptosis in megaloblastic anemia occurs during DNA synthesis by a p53-independent, nucleoside-reversible mechanism. *Blood* 2000;96:3249–55.
16 Ford CE. The interpretation of chromosome counts. *Lancet* 1959;274:567.
17 Powsner ER, Berman L, MacLeod AI. Human bone marrow chromosomes in megaloblastic anemia. *Blood* 1965;26:784–9.
18 Astaldi G, Strosselli E, Airo R, *et al*. [Cytogenetic research in pernicious anemia after treatment.]. *Schweiz Med Wochenschr* 1962;92:1332–3.
19 Kiossoglou KA, Mitus WJ, Dameshek W. Chromosomal aberrations in pernicious anemia. Study of three cases before and after therapy. *Blood* 1965;25:662–82.
20 Keller R, Lindstrand K, Nordén A. Disappearance of chromosomal abnormalities in megaloblastic anaemia after treatment. *Scand J Haematol* 1970;7:478–85.
21 Heath CW, Fliegelman L. Cytogenetic observations in vitamin B12 and folate deficiency. *Blood* 1966;27:800–15.
22 Jensen MK, Friis-Møller A. Chromosome studies in pernicious anaemia. *Acta Med Scand* 1967;181:571–6.
23 Lawler SD, Roberts PD, Hoffbrand AV. Chromosome studies in megaloblastic anaemia before and after treatment. *Scand J Haematol* 1971;8:309–20.
24 Bottura C, Coutinho V. The chromosome anomalies of the megaloblastic anaemias. *Blut* 1968;16:193–9.
25 Chintagumpala MM, Dreyer ZA, Steuber CP, *et al*. Pancytopenia with chromosomal fragility: vitamin B12 deficiency. *J Pediatr Hematol Oncol* 1996;18:166–70.
26 Wollman MR, Penchansky L, Shekhter-Levin S. Transient 7q-in association with megaloblastic anemia due to dietary folate and vitamin Bl2 deficiency. *J Pediatr Hematol Oncol* 1996;18:162–5.
27 Parmentier S, Meinel J, Oelschlaegel U, *et al*. Severe pernicious anemia with distinct cytogenetic and flow cytometric aberrations mimicking myelodysplastic syndrome. *Ann Hematol* 2012;91:1979–81.
28 Forteza BG, Baguena CR. [Cytogenetic analysis of a case of pernicious anemia before and after treatment.]. *Rev Clin Esp* 1963;88:251–4.
29 Goh K. Vitamin B12 deficiency in an 18p-patient. *Arch Pathol Lab Med* 1981;105:164.
30 Haase D. Cytogenetic features in myelodysplastic syndromes. *Ann Hematol* 2008;87:515–26.
31 Braun T, de Botton S, Taksin AL, *et al*. Characteristics and outcome of myelodysplastic syndromes (MDS) with isolated 20q deletion: a report on 62 cases. *Leuk Res* 2011;35:863–7.
32 Wattel E, Laï JL, Hebbar M, *et al*. De novo myelodysplastic syndrome (MDS) with deletion of the long arm of chromosome 20: a subtype of MDS with distinct hematological and prognostic features? *Leuk Res* 1993;17:921–6.
33 Campbell LJ, Garson OM. The prognostic significance of deletion of the long arm of chromosome 20 in myeloid disorders. *Leukemia* 1994;8:67–71.
34 Kurtin PJ, Dewald GW, Shields DJ, *et al*. Hematologic disorders associated with deletions of chromosome 20q: a clinicopathologic study of 107 patients. *Am J Clin Pathol* 1996;106:680–8.
35 Kim M, Lee SE, Park J, *et al*. Vitamin B(12)-responsive pancytopenia mimicking myelodysplastic syndrome. *Acta Haematol* 2011;125:198–201.
36 Bharath V, Hsia CC. Vitamin B12 deficiency presenting as pancytopenia with ring sideroblasts. *Blood* 2015;126:2255.
37 Aitelli C, Wasson L, Page R. Pernicious anemia: presentations mimicking acute leukemia. *South Med J* 2004;97:295–8.
38 Drabick JJ, Davis BJ, Byrd JC. Concurrent pernicious anemia and myelodysplastic syndrome. *Ann Hematol* 2001;80:243–5.
39 Mufti GJ, Figes A, Hamblin TJ, *et al*. Immunological abnormalities in myelodysplastic syndromes I. Serum immunoglobulins and autoantibodies. *Br J Haematol* 1986;63:143–7.
40 Das KC, Herbert V. The lymphocyte as a marker of past nutritional status: persistence of abnormal lymphocyte deoxyuridine (dU) suppression test and chromosomes in patients with past deficiency of folate and vitamin B12. *Br J Haematol* 1978;38:219–33.