Vitamin B$_{12}$ Status after Right Hemicolectomy in Bowel Cancer Patients: A Feasibility Study

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Abstract

Introduction: Terminal ileum resection in patients with Crohn's disease results in vitamin B$_{12}$ deficiency and these patients are routinely given B$_{12}$ supplementation to prevent anemia and neuropsychiatric disease. The effect of right hemicolectomy on B$_{12}$ status in cancer patients has not previously been studied. Total serum B$_{12}$ is an unreliable test. Second-line diagnostic tests measure methylmalonic acid (MMA) and homocysteine (tHcy) are also used. An assay measuring an active form of B$_{12}$ is available for routine use. This observational study investigated whether B$_{12}$ deficiency exists in patients after a right hemicolectomy for cancer.

Methods: Patients (n=28; age range=52-88 years) who had undergone a right hemicolectomy for cancer and been in remission for two years were identified from a database and recruited to an outpatient clinic along with an age-matched control group (n=27; age range=52-89 years). Vitamin B$_{12}$ status in both patients and controls was assessed by the measurement of vitamin B$_{12}$ (total and active), MMA and tHcy.

Results: Concentration of tHcy was significantly higher (p<0.05) in the surgical group (14.0 µmol/L) compared with the controls (11.1 µmol/L). There were no significant differences in total and active B$_{12}$, folate and MMA concentrations between the two groups (p>0.05).

Discussion: Right hemicolectomy for malignancy is associated with an increase in tHcy concentrations although a larger study is required to determine the B$_{12}$ status in post-operative right hemicolectomy patients.

Keywords: Right hemicolectomy; Vitamin B$_{12}$ deficiency; MMA; tHcy; Cancer; Crohn's disease

Introduction

Cancer Research UK reports bowel cancer as the fourth most common cancer for both men and women in the UK, with 41,112 bowel cancer cases in the UK, and 2,272 in Wales in 2013 [1]. Sixty- three per cent of bowel cancer patients had surgical intervention in 2015 [2] with some undergoing a right hemicolectomy with various degrees of the terminal ileum being resected.

There has been no study to investigate vitamin B$_{12}$ deficiency in people undergoing right hemicolectomy for malignancy however those who have had the same procedure for Crohn's disease are prescribed B$_{12}$ supplementation because of recognised deficiencies. Crohn's ileal resections of less than 20 cm in length have variable consequences on B$_{12}$ status [3] although patients who have had more than 20 cm of terminal ileum excised almost invariably have vitamin B$_{12}$ deficiency [4].

Vitamin B$_{12}$ plays a key role in DNA and cell metabolism and long term deficiency can cause severe and irreversible damage. Effects of B$_{12}$ deficiency include fatigue, bone marrow suppression, neurological features including stroke, and risk of cardiomyopathy [5-8]. It has been recognised that B$_{12}$ deficiency can cause symptoms of psychosis, mania, memory impairment, fatigue, irritation and personality changes. Deficiency can be treated with vitamin B$_{12}$ supplementation, which if given for life has the potential to improve well-being and longevity.

Vitamin B$_{12}$ (cobalamin) is bound to two proteins; transcobalamin (TC) and haptocorrin (HC). The transcobalamin-vitamin B$_{12}$ complex, also known as holotranscobalamin (HoloTC), contains the biologically available B$_{12}$ HoloTC mediates the uptake of B$_{12}$ by all cells utilising specific receptors whereas 80% of B$_{12}$ which is bound to HC is metabolically inert [8,9]. Measurement of total B$_{12}$ (cobalamin) in serum by immunoassay is the most widely available test for assessing B$_{12}$ status. However, this test

measures both the active and inactive forms and has a low sensitivity. Currently methylmalonic acid (MMA) and homocysteine (tHcy) are recommended as second line tests if total B_{12} results are in the indeterminate range, as they are considered to be more sensitive tests although tHcy is less specific. Both MMA and tHcy depend on B_{12} as a cofactor for their metabolism. An increase in MMA or total tHcy in folate replete individuals indicates B_{12} deficiency in tissue [7,10]. The metabolically active B_{12} can now be measured by an immunoassay and recent evidence suggests that this is a more reliable marker of impaired vitamin B_{12} status than total B_{12} [7,10]. However, it also recommended that MMA is used as a confirmatory test if the result is in the indeterminate range.

In this present study, we measure both total and active B_{12}, tHcy and MMA in addition to other variables to determine B_{12} status in patients who have undergone right hemicolectomies for cancer and in age-matched controls (controls).

Materials and Methods

Recruitment of participants

Adults treated with a right hemicolectomy for gastrointestinal cancer at Nevill Hall Hospital who had been in remission for two years or more were identified from an internal database and invited to join the study. Potential participants were excluded with the following conditions: already receiving vitamin B_{12} supplementation, pregnant, anemic or unable to give written consent. The study gained ethical approval from the NHS South Central Hampshire B Research ethics committee (REC No 15/SC/0756 PR). All participants gave written informed consent. Twenty-eight participants attended a clinic where blood samples were taken along with twenty-seven age-matched controls (**Table 1**).

Table 1 Descriptive characteristics for all participants (n=55) and for controls (n=27) and patient (n=28) subgroups.

	Total cohort	Controls	Patients	P
n (%)	55 (100%)	27 (49%)	28 (51%)	
Age (years)	75 (52-89)	77 (52-89)	74 (52-88)	0.933
Gender (M: F)	40:15	22:5	18:10	-
Total B_{12} (ng/L)	317 (125-895)[d]	324 (195-895)[b]	295 (125-652)[b]	0.243
Active B_{12} (pmol/L)	65.3 (23.4-257.0)[a]	69.3 (23.4-257.0)	63.7 (27.5-170.1)[a]	0.782
Folate (μg/L)	5.8 (1.5-20.0)[d]	5.8 (1.5-20.0)[b]	5.7 (2.3-16.0)[b]	0.902
MMA (μmol/L)	0.29 (0.16-2.80)	0.28 (0.21-0.81)	0.33 (0.16-2.80)	0.619
Homocysteine (μmol/L)	12.6 (6.5-38.0)	11.1 (6.9-34.5)	14 (6.5-38.0)	0.037
Haemoglobin (g/L)	136 ± 19[a]	133 ± (24)[a]	139 ± (15)	0.256
MCV (fL)	89.0 (79-112)[a]	89 (80-112)[a]	90 (79-104)	0.549
	82 (59-148)[c]	77 (61-119)[b]	84 (59-148)[a]	0.223
eGFR (mL/min/1.73 m^2) (mean ± SD)	73 ± 17[c]	78 ± 15[b]	68 ± 17[a]	0.040
TI Excised (cms)	-	-	80 (60-400)	-
Days post resection			1747 (770-2812)[a]	

Note: continuous variables are expressed as median (range) unless stated otherwise. tHcy, homocysteine; MMA, methylmalonic acid; eGFR, estimated glomerular filtration rate; MCV, mean cell volume. Missing number of samples are shown, a: n-1, b: n- 2, c: n-3, and d: n-4.

Laboratory analyses

Blood samples were collected, transported to the laboratory within 30 minutes and analysed on the same day with the exception of plasma tHcy, plasma MMA, and active vitamin B_{12}, where samples were stored at -80°C for up to one month prior to analysis. For plasma tHcy, samples were separated and frozen within 30 minutes of venepuncture.

Serum total B_{12}, folate, creatinine and tHcy, were analysed on the Abbott ARCHITECT ci8200 analyser (Abbott Diagnostics, Berkshire, UK). Full blood count parameters including haemoglobin and mean cell volume (MCV) were performed by a SYSMEX XE-2100 analyser (SYSMEX, Kobe, Japan). Plasma

concentrations of MMA were determined by GC-MS. Serum active B_{12} was analysed by an Abbott chemiluminescent microparticle immunoassay (CMIA) on the Abbott ARCHITECT ci8200 analyser. Within-batch coefficients of variation (CVs) were 4.1% and 5.3% at concentrations of 15.8 pmol/L and 46.8 pmol/L, respectively. Between-batch CVs were 7.6% and 5.7% at concentrations of 15.8 pmol/L and 46.8 pmol/L, respectively. Glomerular filtration rate was estimated using a four-variable MDRD equation. For the purpose of this study, renal disease was defined as estimated GFR (eGFR) <60 mL/min/1.73 m^2 [11]. Local reference ranges were used for all analytes with the exception of the reference range for active B_{12} [12].

Statistical Analysis

Data were analysed using statistical software (Analyse-It, Leeds UK and Sigmaplot V13 Systat Ltd, UK). Most of the data were not normally distributed and were expressed as median (range) unless stated otherwise. Non-parametric Mann-Whitney U-test was used for comparison between patients and controls. For normally distributed data the unpaired T-test was used. Spearman rank analysis was used to test for univariate relationships between markers of vitamin B_{12} status with clinical variables. A p value of <0.05 was considered statistically significant. Agreement between different markers of B_{12} status was undertaken using the weighted kappa test for agreement: kappa statistic (κ) <0.2 was considered a poor strength of agreement.

Results

Eleven of 26 (42%) patients and 10 of 26 (38%) controls had total B_{12} concentrations within the indeterminate range (180-300 ng/L) **(Table 1).** In comparison, a slightly higher proportion of patients (56% (15 of 27)) and controls (48% (13 of 27)) had active B_{12} concentrations in the indeterminate range (25-70 pmol/L). However, 3 of 26 (12%) patients demonstrated total B_{12} deficiency (<180 ng/L) compared with none in the control group. Conversely, active B_{12} results revealed that 1 of 27 controls (4%) was deficient (<25 pmol/L) compared with none in the patient group. Taken together, there was a high proportion of patients who were not replete in B_{12}; 56% and 53% for active B_{12} total B_{12}, respectively. A slightly lower proportion of controls were not replete in B_{12}; 50% for active B_{12} and 40% for total B_{12}.

Of those study participants (patients and controls) who were not replete in active B_{12} (<70 pmol/L), only 9 of 30 (30%) and 8 of 30 (27%) had raised MMA (>0.42 μmol/L) and tHcy (>16.0 μmol/L) concentrations, respectively. Study participants who were not replete in total B12 (<300 ngl/mL), 4 of 24 (17%) and 5 of 24 (21%) had raised MMA (>0.42 μmol/L) and tHcy

(>16.0 μmol/L) concentrations, respectively. Thus, agreement between total B_{12} and MMA (κ=0.02), active B_{12} and MMA (κ=0.07), tHcy and total B_{12} and tHcy (k=0.01), active B_{12} and tHcy (κ = 0.02) for the diagnosis of B_{12} deficiency was poor in this population. Of those with deficient total B_{12} results (<180 ng/L; n=3 patients), active B_{12} results were all in the indeterminate range or above, one MMA and two tHcy results were elevated, and one tHcy result was at the upper end of the reference range. In contrast, only one control had a deficient active B_{12} result (<25 pmol/L). However total B_{12} was in the indeterminate range and MMA and tHcy concentrations were both normal.

Plasma homocysteine concentration (14.0 μmol/L) was significantly higher in patients compared with controls (11.1 μmol/L) (p=0.037). Ten of 28 (36%) patients exhibited an elevated tHcy concentration (>16.0 μmol/L) compared with 4 of 27 (15%) of controls. eGFR was significantly lower in patients (68 mL/min/1.73 m^2) compared with controls (78 mL/min/1.73 m^2) (p=0.04). However, only seven of the patients and four of the controls had eGFR <60 mL/min/1.73 m^2. There were no significant differences (p>0.05) in total or active B_{12}, MMA, haemoglobin (Hb) and mean cell volume (MCV) between the two groups. The prevalence for abnormal MMA (>0.42 μmol/L) in patients and controls was 21% (6 of 28) and 19% (5 of 27), respectively.

Univariate analyses for the patient subgroup (**Table 2**) revealed that tHcy correlated negatively with folate and eGFR and positively with MMA. However, in the controls, tHcy only correlated negatively with folate (**Table 3**). In patients, total B_{12} only correlated positively with active B_{12} compared with total B_{12} in control participants which correlated positively with active B_{12}, folate and MCV. In patients, active B_{12} correlated positively with total B_{12} and negatively with MMA, and Hb. In the controls active B_{12} correlated negatively with folate and positively with total B_{12}. Due to the small study numbers, the independent effect of variables on B_{12} status was not analyzed.

Table 2 Correlation between vitamin B_{12} status markers and clinical variables in patients (n=28). r_s: correlation coefficient.

	tHcy		Total B_{12}		Active B_{12}		MMA	
	r_s	p	r_s	P	r_s	P	r_s	P
tHcy	-	-	-0.27	0.19	-0.25	0.2	0.43	0.022
Total B_{12}	-0.27	0.19	-	-	0.49	0.01	-0.16	0.42
Active B_{12}	-0.25	0.2	0.49	0.01	-	-	-0.55	0.003
MMA	0.43	0.022	-0.16	0.42	-0.55	0.003	-	-
Folate	-0.74	<0.0001	0.024	0.9	0.24	0.24	-0.45	0.021
Hb	-0.059	0.76	-0.32	0.11	-0.44	0.021	0.32	0.095
eGFR	-0.52	0.0054	-0.25	0.22	-0.073	0.72	-0.12	0.53
MCV	0.19	0.34	0.19	0.34	0.14	0.48	0.044	0.82
TI	0.17	0.38	-0.2	0.33	-0.044	0.83	0.018	0.93
Resection	0.063	0.75	0.098	0.64	0.1	0.61	-0.12	0.54

Table 3 Correlation between vitamin B_{12} status markers and clinical variables in control group (n=27).

	tHcy		Total B_{12}		Active B_{12}		MMA	
	r_s	P	r_s	p	r_s	p	r_s	P
tHcy	-		-0.26	0.2	-0.32	0.099	0.27	0.18
Total B_{12}	-0.26	0.2	-	-	0.51	0.01	0.24	0.25
Active B_{12}	-0.32	0.099	0.51	0.01	-	-	-0.13	0.52
MMA	0.27	0.18	0.24	0.25	-0.13	0.52	-	-
Folate	-0.62	0.001	0.43	0.03	0.44	0.026	0.12	0.57
Hb	0.19	0.34	0.05	0.98	-0.002	0.99	-0.029	0.89
eGFR	-0.36	0.078	0.35	0.096	0.34	0.099	-0.084	0.69
MCV	-0.2	0.32	0.46	0.02	0.36	0.073	0.067	0.74

Discussion

This is the first study to assess B_{12} status in patients who have undergone a right hemicolectomy for cancer. The study has demonstrated that tHcy concentrations were mildly elevated in patients compared with controls. Over twice as many patients had abnormal tHcy concentrations (>16.0 µmol/L) compared with controls. While vitamin B_{12} deficiency results in an elevated plasma tHcy, and is a sensitive marker of vitamin B_{12} deficiency, it is not specific to vitamin B_{12} deficiency and is a nonspecific marker for many diseases including cardiovascular diseases and dementia [13-15]. Recent studies suggest that mildly elevated concentrations of tHcy may be an indicator of atherosclerosis and cardiovascular disease [14]. However, mildly elevated concentrations (<50 µmol/L) frequently occur in the general population and are influenced by many factors, including folate deficiency, vitamin B_6 deficiency poor renal function, hypothyroidism, lifestyle factors (smoking, alcoholism, diet) age and gender, drug therapy and certain genetic polymorphisms. The main predictors of elevated plasma tHcy concentrations are low folate, vitamin B_{12} status or renal impairment [13,14]. Although an inverse relationship exists between tHcy and folate in both patients and controls in this study, all participants, apart from one patient and one control, were defined as folate replete (>2.4 µg/L). Similarly, this study demonstrated an inverse relationship between tHcy and eGFR. Hyperhomocysteinaemia (<50 µmol/L) is known to occur when eGFR falls below 60 mL/min/1.73 m² [16]. Furthermore, it has been demonstrated that renal impairment causes an increase in tHcy and MMA concentrations in an aged population (≥ 65 years) [17]. In the present study, six of the ten patients with raised tHcy concentrations exhibited concurrent impaired renal function (eGFR <60 mL/min/1.73 m²) whereas four patients had normal renal function (eGFR >60 mL/min/1.73 m²). However, the assessment of renal function (eGFR <60 mL/min/ 1.73 m²) in this study is based on a single measurement

and it is recommended that diagnosis of chronic kidney disease is recorded over at least three months [18]. On repeat testing some participants with eGFR <60 mL/min/1.73 m² may exhibit a higher eGFR and vice versa. Notably, the median age of our population is 77 years and thus an effect on renal function is likely which may in turn compromise the interpretation of tHcy and MMA results. The independent effect of renal function on the measurement of tHcy and MMA was not investigated.

The thyroid function and vitamin B_6 status of this group were not investigated and cannot be ruled out as causes. It is possible that these patients also have cardiovascular disease but details of this co-morbidity were not recorded at recruitment and was not part of the exclusion criteria. Further work is necessary to establish the cause of mildly elevated tHcy in this patient group.

Although the study did not show significant differences in active B_{12} concentrations between patients and controls there was an inverse relationship between active B_{12} and MMA within the patient cohort which was not observed amongst controls. Due to the small numbers of participants in this study, the independent effect of variables on MMA and B_{12} concentrations was not explored.

This study highlights the difficulty in defining B_{12} status using various circulating markers. The total B_{12} and active B_{12} results both demonstrate that a large proportion of patients and controls fall within the indeterminate range. According to recent British Journal of Haematology guidelines, patients with a reduced total B_{12} and normal tHcy and MMA do not require any further investigations and are not classified as B_{12} deficient [19]. In the current study, further testing using the recommended second-line investigations, MMA or tHcy, revealed that only a quarter of the study participants with indeterminate active B_{12} results and only a fifth with indeterminate total B_{12} results, may be B_{12} deficient. Although many studies recommend these second line investigations

there is currently not a gold standard test available for B_{12} status. Recently, active B_{12} has been shown to be the best marker of B_{12} status in older people (n=700; mean age=81 years) when compared with red cell cobalamin [7]. Furthermore, in this study the indeterminate range was defined as 20-30 pmol/L and anything above that was defined as normal. If these cut-offs are applied to these data then all study participants would be defined as replete despite having elevated MMA and tHcy concentrations, thereby highlighting the importance of establishing local reference ranges due to variations between populations and methodologies. For the present study, the reference ranges for active B_{12} recommended by the referral laboratory in London have been used, which may not represent the population in South Wales. It would be useful to establish a local reference range for active B_{12}. Thus, further work is necessary before the B_{12} status can be accurately determined in our local population and also in cancer patients who have undergone right hemicolectomy.

Of the participants classified as B_{12} deficient (determined by either total or active B_{12}) none were found to have macrocytosis and/or anemia. Similarly, a Finnish study investigating the prevalence of B_{12} deficiency in an elderly population (n=1048; age range: 65-100 years) demonstrated that anemia or macrocytosis did not predict B_{12} deficiency and only two of their 97 subjects with B_{12} deficiency would have been diagnosed based on these observations [20]. This raises the question of the sensitivity of this approach in the early diagnosis of B_{12} deficiency.

The small sample size in this study does not allow a detailed analysis of B_{12} status as originally anticipated. These results demonstrate that a high proportion of patients (54%) and controls (48%) are not replete in vitamin B_{12}. Whether this is a true representation of the local population needs further investigation and a further larger study would allow a better stratification of study participants into those who are vitamin B_{12} deficient or replete. The population (600,000) served by Aneurin Bevan University Health Board come from both rural and suburban areas, with a mix of socioeconomic status. Those living in rural and lower socioeconomic areas may have a limited access to healthcare and as a consequence are more likely to be vitamin B_{12} deficient.

In conclusion, this study demonstrates that there are subtle differences between the controls and patients who have undergone a right hemicolectomy for cancer. We recommend that vitamin B_{12} status is investigated further using the aforementioned recommended laboratory markers for tissue vitamin B_{12} stores in order to understand how terminal ileum excision alters this essential regulatory system. Replenishing vitamin B_{12} may improve short and long-term outcomes of patients that have had a right hemicolectomy.

Acknowledgements

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