ABSTRACT

Changes in the Methylation Pattern of P53 Gene Promoter in the Megaloblastic Bone Marrow

BACKGROUND: Megaloblastic anemia (MBA) is a type of anemia characterized by decreased number of RBCs as well as the presence of unusually large, abnormal and poorly developed erythrocytes (megaloblasts), which fail to enter blood circulation due to their larger size. Lack of vitamin-B12 (VB12) and/or folate (Vitamin-B9, VB9) with elevated homocysteine is the key factor responsible for megaloblastic anemia. Prior studies have demonstrated the induction of apoptosis in these abnormal under-developed erythrocytes. However, it is not clear whether this apoptosis induction is due to elevated p53 level or due to any other mechanism. Furthermore, it is also not fully known whether decreased vitamin-B12 and/or folate are responsible for apoptosis induction mediated by p53 in pre-erythroblasts.

OBJECTIVES: The objectives of this study are
a) Screening and selection of at least 50 control subjects and 50 megaloblastic cases.
b) Estimation of VitaminB12 and Folate (VB9) levels in control non-megaloblastic anemia samples and megaloblastic anemia cases and correlate with hematological parameters and clinical diagnosis.
c) Measure the expression of p53 in control non-megaloblastic anemia samples and megaloblastic anemia cases using IHC and correlate with VB12 and VB9 levels.
d) Assess whether methylation of p53 promoter has an impact on its expression using methylation specific PCR (MS-PCR)
e) Correlate the levels of p53 expression with levels of apoptosis in megaloblastic anemia (Vitamin B12 and Vitamin B9) and control non-megaloblastic samples.

METHODS: Levels of serum VB9, VB12 and homocysteine in 50 patients suffering from MBA were compared with 50 non-megaloblastic anemia control subjects. Next, we have measured the p53 expression in the paraffin embedded blocks prepared from bone marrow biopsy, using immunohistochemistry, and the expression levels correlated with VB9 and VB12 levels. Next, to determine whether elevated p53 expression in MBA cases is due to defects in the methylation of p53 promoter, a methylation specific-PCR analysis was carried out. In addition, the activity of p53 was measured by assessing the levels of apoptosis in control and megaloblastic anemia cell blocks using TUNEL.

RESULTS: Out of 50 MBA patients 40 (80%) and 44 (88%) subjects had very low VB12 and VB9 levels respectively. In contrast, only 2 (4%) and 12 (24%) non-megaloblastic anemia controls, out of 50 subjects, had low VB12 and VB9 respectively. Correlating with low vitamin B9 and B12, the homocysteine levels were high in 80% cases. But, only 20% non-megaloblastic controls exhibited high homocysteine in plasma. Immunohistochemical analysis for p53 expression showed a significantly high level of expression in MBA cases and no or very low-expression in control subjects. Correlation studies comparing the VB12 and VB9 levels with p53 expression concludes unusually high p53 levels in patients suffering from VB12 and VB9 deficiency induced MBA compared to control subjects. In addition, the expression of p53 is in good correlation with the levels of apoptosis. Interestingly, no
changes were observed in the methylation status of the p53 promoter region either in control subjects or in MBA patients.

**CONCLUSION:** Tumor protein-53 (TP53) is the key transcription factor expressed heavily in the bone marrow biopsies of patients suffering from VB12 and VB9 deficiency induced MBA but not in control subjects. Elevated p53-expression induced apoptosis could be one of the reasons for megaloblasts' shorter survival time compared to normal non-megaloblastic anemia controls. Hence, p53 expression could be used as a surrogate marker for confirming the VB9 and VB12 induced MBA.

**Keywords:** Megaloblastic anemia, P53 gene, VitaminB12, Folate, Homocysteine, Methylation, Apoptosis and P53 expression.