Evaluation and Analysis of Human Folate levels in Pakistani diabetic Population.

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Abstract: In the modern scientific and research era, one of the most rapidly growing areas in biomedical research is treatment of diseases, particularly using advances technologies. The drug discovery and treatment is known to be a valuable field of study and has become one of the most attractive sub-disciplines in clinical medicine for human diseases. In the present research work, the levels of folate specific to human diabetes mellitus in the Pakistani population using advance technology have been identified and characterized. Diabetic patients and same age and sex-matched normal healthy controls were recruited from the Diabetic Clinic, Health Centre, University of the Punjab Lahore, Pakistan were recruited and analysed. Total serum folate levels were estimated and analyzed initially by different standard referred assays protocols at Shaikh Zayed Hospital FPGMI and Shoukat Khanum Cancer Research Hospital Laboratories . All the samples belonging to the control and diabetic groups were then analyzed by standard referred protocol in advance instruments and estimated the levels of Folate which are found to be vary. The discovery of these levels might thus provide a method for early detection of risk for this disease.Variation in the levels of these levels have been reported in other pathological sates. Assessment of the levels of these levels will be helpful in not only early diagnosis but also in prognosis of diabetes mellitus.

Index Terms: diabetes, human population, folate

1. INTRODUCTION

1.1: Diabetes Mellitus: Diabetes is one of the most widely occurring human ailments and the world wide prevalence has risen over the past two decades. According to new publications of some health agencies like World Health Organization (WHO) and International Diabetes Federation (IDF), diabetes becomes an epidemic which is not controlled like other major diseases e.g. cancer, cardiovascular and becomes six leading cause of death by disease worldwide [1]. Humans are not the only species that can develop DM. This disease occurs also in some of animals like dogs, cats and other. It is more common in the developing countries like Pakistan than developed countries. The incidence of this disease in any developed or developing country is difficult to judge. It is quite obvious that the disease is multiplying geometrically more due to genetic and environmental factors [1].

1.2: Folic Acid: Folic acid (also known as folate,vitamin M, vitamin B_9, vitamin B_c(or folacin), pteroyl-L-glutamic acid, and pteroyl-L-glutamate) are forms of the water-soluble vitamin B_9. Folate is composed of the aromatic pteridine ring linked to para-aminobenzoic acid and one or more glutamate residues as in Figure 1 [2]. Folic acid is itself not biologically active, but its biological importance is due totetrahydrofolate and other derivatives

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after its conversion to dihydrofolic acid in the liver.

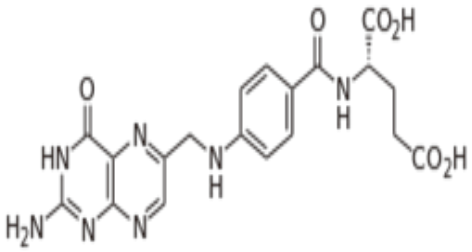

Fig1: Folate Molecule

Vitamin B_9 (folic acid and folate) is essential for numerous bodily functions. Humans cannot synthesize folate de novo; therefore, folate has to be supplied through the diet to meet their daily requirements. The human body needs folate to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in certain biological reactions. It is especially important in aiding rapid cell division and growth, such as in infancy and pregnancy.Children and adults both require folic acid to produce healthy red blood cellsand prevent anemia. Because of the difference in bioavailability between supplemented folic acid and the different forms of folate found in food, the dietary folate equivalent (DFE) system was established. One DFE is defined as 1 µg (microgram) of dietary folate, or 0.6 µg of folic acid supplement. Folate deficiency may lead to glossitis, diarrhea, depression, confusion, anemia, and fetal neural tube defects and brain defects (during pregnancy). Folate deficiency is accelerated by alcohol consumption. Folate deficiency is diagnosed by analyzing CBC and plasma vitamin B_{12} and folate levels [3-4].

1.3. Folic acid Deficiency: A serum folate of 3 µg/L or lower indicates deficiency. Serum folate level reflects folate status but erythrocyte folate level better reflects tissue stores after intake. An erythrocyte folate level of 140 µg/L or lower indicates inadequate folate status. Increased homocysteine level suggests tissue folate deficiency but homocysteine is also affected by vitamin $B_{12}$and vitamin B_6, renal function, and genetics. One way to differentiate between folate deficiency from vitamin B_{12} deficiency is by testing for methylmalonic acid levels. Normal MMA levels indicate folate deficiency and elevated MMA levels indicate vitamin B_{12} deficiency. Patients with megaloblastic anemia need to be tested for vitamin B_{12} deficiency before folate treatment, because if the patient has vitamin B_{12} deficiency, folate supplementation can remove the anemia, but can also worsen neurologic problems. Morbidly obese diabetic patients with BMIs of greater than 50 are more likely to develop folate deficiency. Patients with celiac disease have a higher chance of developing folate deficiency. Cobalamin deficiency may lead to folate deficiency, which, in turn, increases homocysteine levels and may result in the development of cardiovascular disease or birth defects. Folate deficiency is treated with supplemental oral folate of 400 to 1000 µg per day. This treatment is very successful in replenishing tissues, even if deficiency was caused by diabetes and other diseases [5-7].

1.4. Folic acid and diabetes mellitus: Folate has been implicated in numerous disease states and

involve in the prevention of neural tube defects. However in the past decade folate deficiency or disturbances in folate metabolism have been associated with neurological degeneration , cancer and CVD, which is the subject of this review. Folate metabolism is closely linked to that of homocysteine . Elevations of plasma total homocysteine (tHcy) may occur due to genetic defects and or an inadequate status of the vitamin cofactors needed for these reactions associated with plasma folate status even within the range that is considered to be normal. Folate intake is also negatively correlated with plasma homocysteine, reaching a plateau at a daily intake of _400 _g/day. However, the associations between vitamin B12 and B6 status with homocysteine are weak. Folate can lower plasma homocysteine levels safely and effectively.

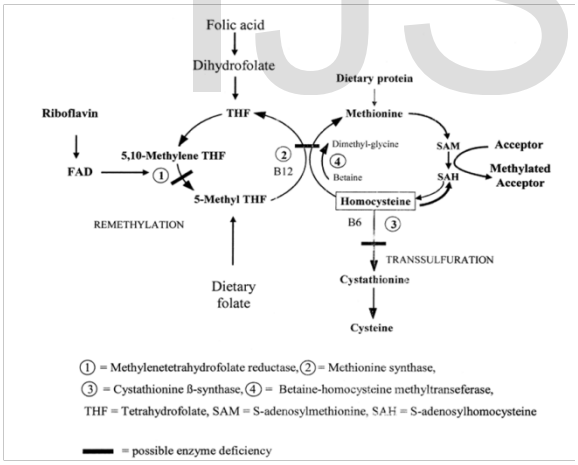

Fig 2: Metabolism of Folic acid and Homocysteine (adapted from

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B12 and B6 have only modest homocysteine lowering effects. There is evidence that riboflavin (vitamin B2), as the precursor to flavin adenine dinucleotide (FAD), the cofactor for MTHFR, is also a determinant of homocysteine concentrations . Folate status is the most important determinant of tHcy in the general population, therefore, the association between folate status and CVD would support the concept that low level of folate is a risk factor as in Figure 2 [8-10].

Diabetes mellitus (DM) is a metabolic and multifactorial syndrome with disordered metabolism and hyperglycemia. The world diabetic population is currently 285 million (IDF 2009) and projected to reach 438 million by the year 2030 (IDF 2009) of which 95% are type 2. Cost of managing diabetes in the U.S alone is more than 44 billion dollars annually. Globally, morbidity and mortality figures reflect some 3.2 million people die of diabetes each year and 6 people die of diabetes related ailments each minute (WHO 2003). It ranks second in causation of retinopathy and nephropathy worldwide. Diabetics have 2-4 times more heart disease at an earlier age and a two times higher risk of stroke than non-diabetics Pakistan ranked 6th in a list of 10 nations with the highest number of diabetics reaching 5.2 million in 2004 [11-16].

1.5. **Epidimiology of diabetes:** In case of persistence of the same trend, Pakistan will rise unfortunately to 4th position with 14.5 million diabetic patients by the year 2025. For Pakistan special significance only 1% spent on health care budgets, declining economic conditions, inadequate diet and resources for treatment and overpopulation

have lead to an explosion in type 2 diabetic population [17-18]. Research into and requirement for a safe , easily accessible, cost effective drug which could supplement diabetes treatment and delay its microvascular and macrovascular pathologies was the need of the hour. The level of folate should be decrease in the diabetic patients as compared with that of normal person. This showed that deficiency of folate will results into other cardiovascular diseases and it act as risk factor. Treatment with high doses of folic acid will lowers the plasma total homocysteine significantly but also improve any of the associated cardiovascular risk factors. Short and Long-term clinical trials should be conducted to determine whether high-dose vitamin treatment will diminish the increased morbidity and mortality associated with cardiovascular disease in patients with diabetic mellitus.

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