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Homocysteine Related Vitamins in Smoking and Non-Smoking Secondary polycythemia

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ABSTRACT

The amino acid homocysteine is an intermediate in metabolic pathways between the common amino acids methionine and cysteine. The vitamins folate ,and B12 ,and to a lesser extent B6, are needed for its proper functioning .Thus ,low availability of folate ,B12,or B 6 can block the path way and lead to a buildup of homocysteine.

This study was conducted to determine the levels of homocysteine, lipid peroxidation and vitamins in smokers and non- smokers subjects which they come to the blood bank in orders to eliminates the surplus blood (secondary polycythemia). The study population consisted of 40 male patients divided in to two groups ; 30 smokers and age matched non-smokers 10 subjects. The mean systolic and diastolic blood pressure values and haematocrit were found to be non-significant . Haemoglobin blood values was found to be significantly($p<0.05$) higher for smokers than for non- smokers .Serum Vitamin A Significant decrease($p<0.05$)in smokers subjects . Vitamins B12, vitamin E and folate found significantly decrease ($p<0.1$) in smokers as compared to non-smokers. No significant values in B-carotin vitamin B6 and vitamin C in serum between smokers and non- smokers subjects . There was a significant value($p<0.05$) of lipid peroxidation in non smokers than smokers subjects. Serum levels of homocysteine in normal value in both groups .

Key words : homocysteine, lipid peroxidation, vitamins, polycythemia

INTROD UCTION

The amino acid homocybbsteine is an intermediate in metabolic pathways between the common amino acid methionine and cysteine .It is called homocysteine because it is a chemical homologue of cysteine . That is , it is like cysteine (the – SH group containing amino acids) , but with an extra methylene (CH2) in its carbon chain. The reaction in the metabolic path that includes homocysteine are very dependent on vitamins as cofactors that helps in the reactions(1). The vitamins folate and B12, and to a lesser extent B6 , are needed for its proper functioning . Thus , low availability of folate , B12 , or B6 can block the pathway and lead to a build – up of homocysteine. There are also heritable detects in the pathway – particularly of the MTHFR enzyme – that can raise homocysteine levels . In renal failure , homocysteine is often elevated ,but as homocysteine is not primarily cleared by the kidney the mechanism of elevation is uncertain in many renal failure patients(2). Several lifestyle factors may influence homocysteine levels, smoking , high consumption of alcohol , and inadequate nutrition , all arefactors that have a considerable impact on health. Connection between lifestyle and other factors that increase homocysteine and its complications attributable to a disturbed homocysteine metabolism have not yet been given much focus . However , many interesting observations support such a connection . Ever body

has a certain amount of this within their body , it is also though that low levels of thyroid hormone , kidney disease and psoriasis can lead to increases the levels of homocysteine (3).

Elevated levels of homocysteine in the blood may be associated with increased risk of heart attack , strokes , blood clots , hardening of the arteries , and possibly Alzheimer s disease .There is also much focus on the association between carcinogenesis and deficiency of vitamins involved in the homocysteine metabolism of primary folate(4) .

Symptoms of elevated level of homocysteine in the blood include increased tendency of blood clotting , deep vein thrombosis and pulmonary embolism .Insufficient amounts of folate , vitamins B6 and B12 are associated with increased levels of homocysteine in the blood . When your body has these vitamins it can quickly change homocysteine in to compound that are safe for your arteries(5) . Our study is to determine the levels of homocysteine , lipid peroxidation and vitamins profile in patients with secondary polycythemia .

MATERIAL AND METHODS

Venous blood samples were taken from 40 subjects (10 non smokers mean aged 29.9 year and 30 smokers mean aged 34.3 year) ,which they come to the blood banking in order to eliminate the surplus blood followed up during three months (from January until april 2011) . Ten ml of venous blood was collected from each subject by vein puncture and 2.5 ml transferred to EDTA tube for haematological parameters and the rest of blood was centrifuged in 3hrs of collection at 3000 rpm for 10 minutes. The serum was used for the measurement of MAD in the serum ,which is an index of lipid peroxidation , is based on the reaction with thiobarbituric acid (TBA) to form a colored chromophore (TBA2-MDA adduct), a ccording to the method of Buege and Aust (1978)(6). The pink colored chromophore formed was measured spectrophotometrically at wave length 535nm . The concentration of MDA was measured using the molar absorbtivity coefficient of 1.56x 15 -6 M-1 cm-1. The result were expressed as umol MDA/L. Vitamin C was detected by high- performance liquid chromato graphy with electrochemical detection according to Kutnink et al.(7) with a supelco C18 column (250mm x 4.6 inner diameter) and a supelco C18 guard column (20mm X 4.6 inner diameter)(8). Vitamins A and E were measured , after extraction with ethanol and hexane , by high- performance liquid chromatography with UV detection at 280 nm 17 with a waters symmetry C8 column (150mmx 4.6 inner diameter). B-carotene was detected after extraction with hexane – dichloromethane (5:1) by high performance liquid chromatography at 450 nm using a supelco C18 (250 mm x 4.6 inner diameter).

Serum's homocysteine level was determined with high performance liquid chromatography . Serum folate and vitamin B12 levels were measured by using an immune assay to determine the vitamin deficiency. Folate deficiency was considered present when the serum folate level was less than 11.4 n mol/liter and the homocysteine level was higher than 13.9 m mol/liter . Vitamin B12 deficiency was defined as a level less than 258 pg mol/ liter (9).

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STATISTICAL ANALYSIS

All data are expressed as the mean value . Statistical analysis of the results were performed using ANOVA table, Duncan S test was used to compare the mean results between the non-smokers and smokers subjects of secondary polycythemia.

RESULTS AND DISCUSSION

Table (1) show an increased level of haemoglobin between non- smokers and smokers subjects ($P<0.05$) , while non-significant differences in haematocrit value. Haemoglobin can accelerate lipid peroxidation by two mechanisms . Firstly the reaction of heme ring with equimolar concentrations of H2O2 produces, an oxo-iron species , probably ferryl , and its formation can lead to stimulation of lipid peroxidation .how exactly this activated heme can interact with free fatty acids or fatty acyl side chains in membranes while still remaining bound to the protein has yet to be established . Secondly, excess H2O2 can cause degradation of ions that are capable of stimulating .OH production and lipid peroxidation . Heme , released from damaged heme proteins ,is also a powerful stimulator of peroxidation (10). No significant difference in blood presser between non –smokers and smokers subjects of secondary polycythemia .

Table (1) Haematological Parameters of non –smokers and smokers patients

Parameter	Normal value	Non-Smokers	Smokers
		MEAN	'MEAN
Number		10	30
Age (year)		29.9	34.3
Haemoglobin (gm /dl)	15	15.37	15.93**
Packed cell volume %	43-49	46.8	48.63
Blood pressure (mm/ Hg)	12/8	13.3/8.6	13.5/ 8.8

**$p<0.05$

The determination of homocysteine ,reactive oxygen species concentrations ,and total antioxidant capacity provide a rational basis for correcting the increased oxidation associated with disease progression (11).

The clinical role of oxidative stress was normaly investigated by measuring reactive oxygen species (ROS) , homocysteine , vitamins A , E ,C ,B-carotin , B12 and Folate .

significant differences ($p<0.05$) in the level of malondialdehide (MDA) between non smokers and smokers, while homocysteine level was in a normal range .

We found low levels of serum vitamin A ($p<0.05$) in smokers compared with non smoker subjects and also in vitamin E ($p < 0.1$), suggesting a protective role of these nutrients . Same results found in vitamin B12 and folate ($P <0.1$) . There are two major roles for vitamin B12 ; synthesis of methionine from homocysteine ; and conversion of methylmalonyl coenzyme A to succinyl coenzyme A. There are five major metabolic roles for folate : (a) serine and glycine metabolism ; (b) histidine catabolism ; (c) thymidylate synthesis ; (d) methionine synthesis; and (e) purine synthesis. A deficiency of either vitamin B12 or

folate can lead to megaloblastic anemia(2) . The conversion of methylmalonyl coenzyme A to succinyl coenzyme A requires vitamin B12 ,there for a deficiency of vitamin B12 causes increase in the concentration of methylmalonyl (4) . Folic acid ,the synthetic form of the B vitamin folate work primarily in the brain and nervous system and is necessary for the synyhesis of DNA , production of red and white blood cells and of norepinephrine and serotonin in the nervous system . Folic acid also aids in the elimination of the amino acid homocysteine from the blood , a breakdown product of animal protein (methionine), actually that contributes to the heart attacks(9).

Table (2) Serum levels of homocysteine, vitamins and MDA in patients with secondary Polycythemia

Parameter	Non-smokers	Smokers
Number	10	30
Homocysteine(m mol / L)	9.178	8.134
Vitamin A (ug/dl)	57.18	46.63**
Vitamin E (umol / l)	12.66	10.08*
Vitamin C (mg/dl)	0.97	1.04
Vitamin B12 (ng/ml)	8.55	5.9 *
Folate (ng/ml)	4.85	3.17*
B-carotin (Ug/dl)	66.47	56.52
MDA (umol/L)	3.167	2.17**

*significant ($p<0.1$) ,** significant ($p<0.05$)

Measurement of peripheral antioxidant is considered an appropriate way of looking at oxidative stress in various disease states in humans (12).

Our results indicate that there is a decrease in antioxidant defense mechanism in smokers when compared to non-smokers subjects . The human body has inherent synergistic and multilevel defense mechanism which comprise of two major classes of cellular against reactive oxygen species (13) . Free radical scavenger enzymes namely Superoxide dismutase, Catalase and Glutathione peroxidase represent the enzymatic part . The non enzymatic part includes a large number of natural and synthetic antioxidant compounds (Glutathione and Vitamins) that have the ability to inhibit oxidative stress by scavenging the highly destructive free radicals.

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العلاقة بين Homocysteine والفيتامينات لدى المدخنين وغير المدخنين لدى مرضى زيادة نسبة الدم الثانوي

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الخلاصة

حامض اميني اساسي يستطيع جسم الانسان تخليقه من الحامض الاميني الاساسي methionine ، يدخل هذا الحامض المسارات الايضية لغرض انتاج الحرارة والطاقة ويحتاج الى فيتامينات Folate , B12 and B6 كعامل مساعدcofactor في المسار الايضي. انخفاض مستوى هذه الفيتامينات في الدم يؤدي الى انخفاض في تأيضه مما يرفع من مستواه في الدم ، ارتفاع مستوى homocysteine في الدم يتسبب في امراض جهاز القلب الوعائي ، الفشل الكلوي ، الجلطة الدماغية نتيجة الضرر الذي يحدثه هذا الحامض بالوعاء الدموي (يمثل بجلافة sand paper للوعاء الدموي).

اجريت هذه الدراسة لتحديد مستويات هذا الحامض في مصل الاشخاص الذكور من المدخنين ومقارنتها بغير المدخنين المراجعين الى مصرف الدم في بعقوبة لغرض التخلص من زيادة نسبة الدم (Secondary polycythemia)،وقياس الضرر الحادث من خلال قياس الشحوم المؤكسدة باجراء قياس(MDA) manodaldehyde الذي يعتبر كمقياس للضرر الحادث ،بالاضافة الى قياس تراكيز بعض الفيتامينات في المصل.

اشتملت الدراسة على ٤٠ شخص من الذكور المصابين بزيادة نسبة الدم (٣٠مدخن ، ١٠ غير مدخن) ، تم تدوين العمر، قياس ضغط الدم ، قياس خضاب الدم وحجم الخلايا المرصوصة . لم تظهر النتائج وجود فروقات معنوية في حجم الخلايا المرصوصة وضغط الدم ولكنها اظهرت فروقات معنوية في خضاب الدم عند مستوى احتمالية ($P < .05$).بين المدخنين وغير المدخنين . أما إختبارات مصل الدم فقد أظهرت فروقات معنوية عند مستوى احتمالية ($P<.05$) في مستويات فيتامين A وعند مستوى احتمالية ($P<0.1$) في مستويات فيتامينات Folate , E and B12 بين المدخنين وغير المدخنين ،بينما لم تظهر أية فروقات معنوية في B- Carotin , Vitamin B6 and Vitamin C بين المجاميع . وهناك ارتفاع معنوي عند مستوى احتمالية ($P < .05$) في الشحوم المؤكسدة بين المجاميع ، أما مستويات homocysteine فهي ضمن القيم الطبيعية .

الكلمات المفتاحية : homocysteine – الفيتامينات – اكسدة الشحوم – زيادة نسبة الدم