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Hyperhomocysteinuria with urinary incontinence in psoriasis patients of West Bengal, India

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ABSTRACT

Psoriasis is a most prevalent hyperprolifetive skin disorder affecting about 2-4% of the population world wide. Contributing factors for development of different types of psoriasis are genetic, environmental and immunological factors. Diseases characterised by hyperproliferative disorders are mostly associated with the aminoacidopathies due to mutation or presence of polymorphically distributed gene of the metabolic pathway affecting the metabolism. Diseases treated with Antihistaminic, anti proliferative or anti mitotic agents and antihypertensives may develop aminoacidopathy particularly homocysteinemia or homocysteinuria. Cancer and psoriasis vulgaris are also associated with increased homocysteine concentration in plasma. Recently association between methylenetetrahydrofolate reductase (MTHFR) gene polymorphism and psoriasis vulgaris has attracted great interest amongst researchers worldwide. We are therefore interested to find out the correlation with aminoacidopathies (if any) with plaque psoriasis, particularly, methionine, cysteine and homocysteine balance.
34 plaque psoriasis patients and 30 age matched control were chosen from tertiary referral unit of dermatological clinic of Calcutta Medical college Hospital and subjected to amino acid profiling in deproteinized plasma and urine samples. HPLC analysis revealed a significantly increased amount of homocysteine in both plasma and urine compartment of the body of plaque psoriasis patients in comparison to control.

Key words: Psoriasis, amino acidopathy, hyperhomocystenuria, hyperhomocystenemia, Methelene tetrahydrofolate reductase, trans -sulfuration/transmethylation pathway.

INTRODUCTION

Psoriasis is a chronic papulo-squamous disorder of the skin, mostly affecting the 2% population of tropical countries and characterized by well demarcated red scaly plaques. It is the inflammatory disorder of the skin and shows all of the signs of inflammation. Hyperproliferation of skin developing scaly appearance over the epidermis is the signature feature of psoriasis [Schofield & Ralston 2010]. Research reveals that it is associated with abnormalities in several genetic loci mostly present in chromosome number 1, 20, 3, 17 and 6. So far, a total of 16 potential susceptibility loci located on 14 chromosomes have been identified by genome-wide screening [Tsoi et al. 2012]. Among these, seven suspected loci are now designated as PSORS 1 to 9 (Schofield & Ralston 2010, Bowcock & Barker. 2003).

The pathophysiology of psoriasis characterized by abnormal turnover of skin cells with a high proliferative index which is associated with altered genetic equilibrium of the genes responsible for maintaining mitotic index [Schofield & Rees 2010; Paige 2010]. Hyperproliferative keratinocytes with a grossly increased mitotic index and an abnormal pattern of differentiation, causes retention of nuclei in the stratum corneum. Infiltration of T lymphocyte in the upper dermal layer is associated with it. Association with different genetic loci have been

implicated psoriasis as a complex genetic and metabolic disorder. Polymorphisms of interleukin genes are also associated with disease like psoriasis.

Diseases characterized by hyperproliferative disorders, different types of cancers and different types of immunological alteration are shown to be associated with amino acidopathies. It has been reported from a study conducted in Italy that serum homocysteine level is significantly high in psoriatic patients in comparison to control population [Brazelli et al. 2010]. Malerba and co-workers have shown that plaque psoriasis is associated with hyperhomocysteinemia [Malerba et al. 2006]. In another study conducted in Turkey revealed that plasma homocysteine level positively correlate with the psoriatic severity index (PASI score) of psoriasis patients. In those patients serum homocysteine level is inversely correlated with serum folic acid level [Cakmak et al. 2009].

Antimitotic drug, Methotrexate treatment and treatment with immunosuppressive drugs also cause amino acidopathies characterized by hyperhomocysteinnemia, an abnormality of homocysteine metabolism. Besides, antihistaminic drugs, anti hypertensive drugs, cholesterol lowering agents and some classes of anti diabetic agents are also known to cause abnormal homocysteine metabolism and give rise to hyperhomocysteinemia [Krishnamoorthy & Mohan 2012]. Further, people with hyperproliferative disorders like different types of cancers and diseases like types of psoriasis have been shown to induce hyperhomocysteinemia with and with out treatment [Malerba et al. 2006, Cakmak 2009]. Psoriasis is often treated with anti mitotic agents with immunosuppressive medicines which can also induce hyperhomocysteinemia [Pal & Arora et al.2012] . Therefore we hypothesizing that psoriasis in our country with in a particular ethnic group (Eastern Indian Population, from State West Bengal, Bengali Population) may be associated with aminoacidopathies, more precisely with abnormal homocysteine metabolism. All though a few reports available to focus amino acidopathies in some types of psoriasis still those reports are not enough to point out those aminoacidopathies as biomarkers for psoriasis. Searching for a specific biomarker for identification of psoriasis therefore should be the primary focus right now. Characteristic dermal plaques with subsequent desquamations are the best possible route to detect psoriasis. Psoriasis is linked with the mutations or polymorphisms in a numbers of genetic loci. Alterations, which are frequently associated with genetic cause may give metabolic error to the body, may be reflected by altered amino acid profiling. A numbers of diseases are now identified having altered amino acid profile including inborn errors of metabolism diseases (IMD) which are also classified according to their amino acidopathies. Urine is a choice of body fluid to use as a matrix for biomarker identification in our work. Because it is easy to collect and non-invasive in collection nature. Therefore we are interested to find out the biomarker of psoriasis from urinary amino acid profiling.

Hyperhomohomocysteinemia which is thought to be associated with psoriasis (as it is a hyper proliferative disorder) is generated by abnormal metabolism of homocysteine. This is caused by mutations of the enzymes involved either in homocysteine generating pathway or remethylation pathway. The key enzymes for trasmethylation /transsuferation (remethylation of methionine with generation of cysteine from homocysteine) are Cystathione β Synthase (CβS), Methelene Tetra hydro Folate Reductase (MTHFR) and Methylene Synthase (MS).

Alteration to any of these enzyme activity or mutation to any of these enzymes may contribute aminoacidopathies [Arora 2012, Engbersen et al. 1995].

It has been reviewed that a particular type of psoriasis is associated with mutation (SNP) in a particular site of MTHFR with a transition at 677bp (677C-T transition) which results in substitution of alanine to valine to form a thermolabile variant of the enzyme with a reduced catalytic activity [Liew et al.2012). This mutation is thought to be responsible for inappropriate methionine metabolism and development of hyperhomocystenemia. Researchers also categorize another MTHFR mutation in a different position involving 1298 A- C transition with a substitution of glutamate to alanine. This substitution is nonsence in nature and causing no alteration in the amino acid profile of individual [Liew et al.2012].

We hypothesized that if psoriasis patients have MTHFR polymorphism (677 C-T) then they may develop abnormal amino acid profile with an altered methionine metabolism which may be reflected as plasma and urinary amino acidopathies. In the current text we are emphasising on the urinary amino acid profiling with the measurement of both plasma and urine amino acids.

EXPERIMENTAL SECTION

34 psoriasis patients were chosen from a tertiary referral clinic of School of Tropical Medicine and Calcutta Medical College Hospital. All of the patients were from same age group (25-40 years of age) with a mean age of 33 years. All of the participants were from same socio economic status and from south and north 24 parganas of the southern portion of West Bengal, a state in the republic of India. Patient selection was done by dermatologists attending the

dermatological clinics in the said Govt. hospital. Clinically diagnosed cases of psoriasis were chosen for the study who were not receiving any immunosuprresive agents or any oral medication for their skin disorders including methotrexate. All of the 34 psoriasis patients were representing plaque psoriasis with dry silvery white scales over the skin.

Normal healthy age and sex matched persons having no biological relation to psoriasis patients served as control. Written informed consent were taken from each participants before include them in the study population.

All the subjects included in this study was categorised into two groups, case (psoriasis patients, (N=34) and control (N=30).

2 ml of EDTA anticoagulated blood and 3 ml of blood with out any anticoagulant was collected from each participant. Morning void urine was also collected from each participant in sterile urine container with protease inhibitor. The biological samples were transported into the ice bag to the department of Physiology, Presidency College for further study.

The plasma and urine sample was used for HPLC where as the serum was used for biochemical assay for total protein, albumin, globulin, urea and creatinine estimation.

Each of the psoriasis patient studied was given a PASI score according to their severity of disease. The score of PASI with in a range of 1to 23 signifies mild, 24 to 50 signifies moderate and above 50 signifies severe skin lesions. Our control subjects have a PASI score '0'.

Biochemical assay: Total protein (by Bradfoard method), Serum albumin (by Bromocresol Green Method), serum urea (Modified Berthelot Method) and serum creatinine (by JAFF's reaction) were estimated from patient's group and control group.

Deproteinization of Plasma and Urine Sample: 100µl of each plasma and urine samples were mixed with β-mercaptoethanol and allowed to stand for 5 minutes at room temperature (25º C) and then purified and precipitated by methanol. After 15 min incubation at ice the samples were centrifuged at 5000 rpm at 4º C for 20 min. The protein free supernatants from plasma and urine were then subjected for total amino acid estimation.

Assay of total amino acids: Total amino acids content of protein free supernatants of plasma and urine were estimated by modified dinitrophenyl (DNP) derivatization method (17). Methanol extracted (100 µl) plasma or urine and the reference standard (equimolar mixture of glutamate:glycine) was made up to 250 with 80% (v/v) methanol. Equal volume (250 µl) of borate buffer was added to each of the tubes, followed by the addition of 0.5 ml of DNFB reagent. The cocktails (sample+ methanol+ borate buffer) were then incubated at 45°C for 30 minutes and allowed to cool at room temperature. After adding 1 ml of 0.25 M HCl to each of the tubes and thorough mixing, OD was taken at 420 nm.

Ascending Paper Chromatography of Urine Samples: The urine samples were subjected to paper chromatography in which the chromatography chamber was pre-saturated by for 16 hrs by butanol-acetate solvent(butanol: acetic acid: water :: 4:1:5). The liquid phase used in chromatography was also butanol acetate solvent. An equimolar mixture of amino acids(2mg/ml) were used as reference. Urine samples to be separated on the basis of their partition coefficient were loaded on to the lower margin of the paper by a capillary tube. The chromatography was performed for 9 hrs in air tight chromatography chamber. After completion of the run, the moist paper was brought out and air dried. The colour was developed by ninhydrin (0.1% ninhydrin solution) reaction and subsequent incubation of the paper at 110ºC. A reference was prepared to subject to the chromatography by using equimolar mixture of amino acids (2mg/ml). This was used as standard.

The value obtained from densitometric analysis was calculated by following equation. All of the values obtained was normalised using the value of reference value (value of standard).

$$\frac{\text{Intensity of colour in psoriasis} - \text{intensity of colour in standard Homocysteine}}{\text{Intensity of colour in control} - \text{intensity of colour in standard Homocysteine}}$$

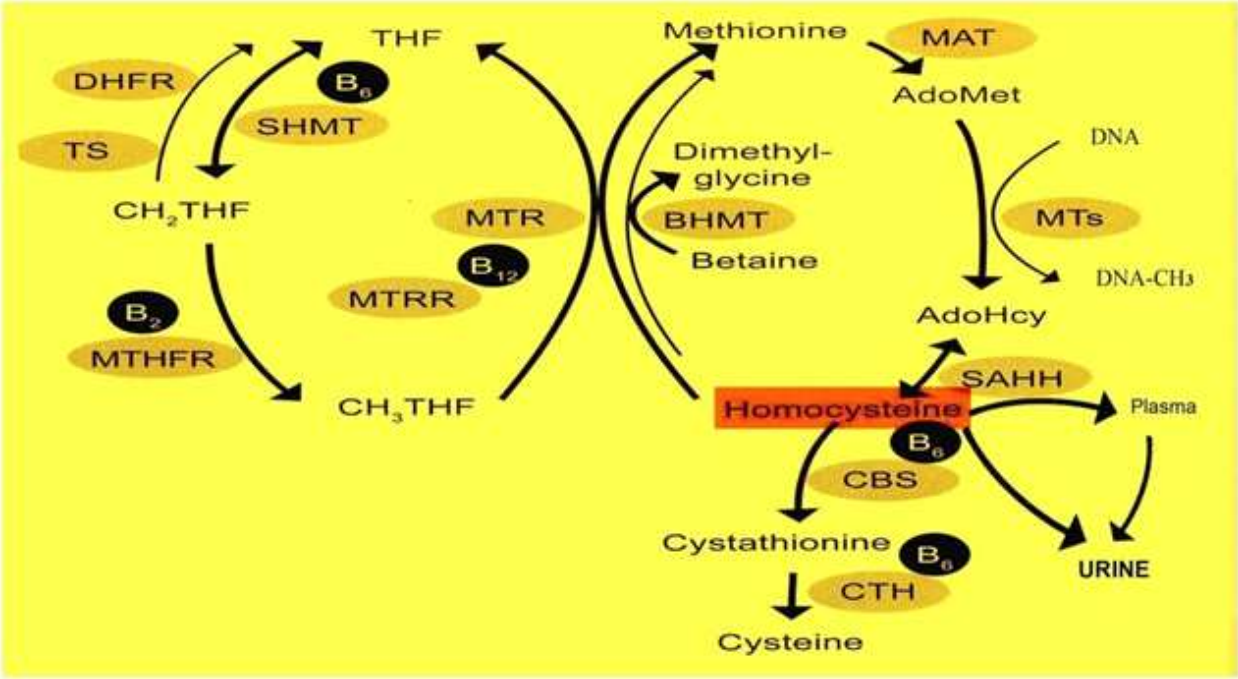
Profiling of amino acids present in Plasma and Urine by HPLC:
For HPLC, an equimolar concentration (0.1mM) of cocktail of amino acids and sample sup (plasma /urine) extracted in methanol were mixed with 50 µl Sodium Borate buffer (0.5M, pH 10.5 containing iodoacetate (0.2M) followed by 25 µl ortho- pthalaldehyde (OPA) reagent for derivatisation. Derivatized sample was made up to 1 ml

by addition of 825 µl of start eluent (4:2 v/v mixture of acetate buffer, pH 6.8 and methanol). OPA derivatized amino acid mixture were then injected to HPLC system equipped with 20 µl injection loop and C18 column (25 cm) fitted with a guard column (1 cm) housed in an incubator oven set at 40° C constant temperature. Individual amino acids were separated by reverse phase and methanol with a 1 ml /min flow rate. Resolution of amino acids derivatives were monitored through fluorescence detector with excitation and emission spectra set at 330nm and 450nm respectively.

RESULTS

We have found hyper proteamia with reduced serum albumin and increased globulin level in psoriasis patients. Serum urea showed an increase and creatinine showed a decrease in patient's group. All the psoriasis patients have a decreased urinary pH with an increased urinary homocysteine (table 1 and table 2). Paper chromatography of the urine samples from psoriasis patients and from normal individuals have shown that there was an increased intensity of the developed colour after ninhydrin reaction in patient's group at the respective spot for homocysteine. We performed densitometric analysis (KODAK Digital Science ID) from every chromatography paper having spots for different amino acids with the reference scale. The data obtained showed a significant increase in the value of homocysteine in every psoriasis patients in comparison to control group (table 2). The result indicates a significantly increased urinary output of homocysteine in psoriasis patients in comparison to control.

Figure 1: Transmethylation- transsulfuration pathway: reflecting the methionine, cysteine, homocysteine metabolism



Note: Abbreviations used:
Ado Hcy- S-Adenosyl homocysteine, Ado Met- S- Adenosyl Methionine, B2-Vitamin B2, B12- Vitamin B12, B6- Vitamin B6, BHMT- betaine-homocysteine S-methyl transferase, CBS-Cystathione beta Synthase, CH2THF- 5,10, methylene tetra hydro folate, CTH- Cystathionase, DHFR-dihydrofolate reductase, MAT- methionine adenosyl transferase, MTs-AdoMet dependent methyltransferase, MTHFR- methelene tetra hydrofolate reductase, MTR- methionine synthase, MTRR- methionine synthase reductase, SAHH- S adenosyl homocysteine hydrolase, SHMT-serine hydroxymethyl transferase, THF-tetra hydro folate, TS- thymidylate synthetase.

Table 1: Median values of Biochemical parameters in control and psoriasis patients

Median value	Total Protein	Albumin	Globulin	Albumin: Globulin	Urea	Creatinine	Urinary pH
Control	6.443	2.575	3.616	0.74	13.75	7.95	6.9
Patient's group	7.218	1.615	5.518	0.28	14.05	6.15	5.5
X2 value	7.2	4.811	3.92	3.99	0.002	0.9	4.18
P value	P<0.05	P<0.05	P<0.05	P<0.05	p>0.05	p>0.05	P<0.05

On the basis with this preliminary data we have performed HPLC with urine and plasma samples collected from each study subject in both patient and control group. HPLC results also showed significant increase in the homocysteine concentration in patient's group in comparison to control group (table 3). Among those 34 subjects 21 showed urinary incontinence. All of those 21 subjects had high PASI score (degree of severity index for psoriasis) which signifies that degree of urinary homocysteine increases with the severity of psoriasis (data not shown). Further higher the urinary homocysteine level there was a severity in urinary incontinence (clinically correlated by patient reviewing).

Table 2: Densitometric value of paper chromatographic expression of urinary homocysteine concentration of control and psoriasis patients

	Median value of homocysteine
Control	0.8663
Patient's group	1.491
X2 value	4. 67 P<0.05

Table 3: Median values of plasma and urine amino acid concentration from HPLC analysis studied in patient's group and control group
(Nd: not determined)

Amino Acid	**Plasma µmol/L**		**Urine µmol/mMol Creatinine**	
	Normal Median value	**Psoriasis Median value**	**Normal Median value**	**Psoriasis Median value**
Glycine	260	256	149	153
Alanine	330	321	49	56
Valine	156	139	13	11
Leucine	84	93	6	4
Isoleucine	57	63	5	4
Serine	99	106	67	53
Threonine	122	107	27	21
Aspertate	19	23	8	7
Glutamate	36	31	2	Nd
Asparagine	53	60	17	6
Histidine	70	63	96	23
Arginine	75	74	8	Nd
Lysine	149	154	38	21
Ornithine	67	73	6	Nd
Phenylalanine	60	61	9	8
Tyrosine	54	49	18	21
Tryptophan	58	51	Nd	Nd
Cysteine	211	201	13	Nd
Homocysteine	10	56	71	29
Methionine	27	29	11	09
Taurine	85	81	107	103

DISCUSSION

Cellular homocysteine is metabolised by a balanced correlation between transmethylation and transsulferation pathway where both are linked at more than one points and facilitate inter conversion of substrates. The enzymes involve, maintain the reaction sequence and balance between these two interlinked pathways are Cystathion β synthase (CβS), 5-10 Methylene tetrahydrofolate Reductase (MTHFR) and Methionine Synthase (MS). As psoriasis is associated with hyperhomocystemia people hypothesized that there is a metabolic block in the homocysteine to cysteine conversion which enzymatic step is catalysed by CβS.

Traditionally hyperhomocysteinemia and hyperhomocysteineuria commonly found in people with vit B6, folic acid and B12 deficiency. Other causes of hyperhomocysteineuria are genetic defects. Homocysteine accumulation may be caused by a metabolic block in either the degradation of homocysteine to cystathionine or the remethylation of homocysteine to methionine (fig. 1). The classic form of severe hyperhomocysteinemia is caused by CβS enzyme deficiency [Pal & Arora et al 2012].

This enzyme catalyzes the formation of cystathionine from homocysteine and serine.

Polymorphism or any mutation in CβS gene can affect the homocysteine metabolism pathway as it is the first rate limiting enzyme in this pathway. CβS deficiency results from genetic mutations (1278 T and G307S) and more than 150 point mutations, insertions and deletion. Although in most of the cases with classical hyperhomocysteineuria there is a strong association with G307S mutation, till date no reports available to support any association with the mutations of CβS in psoriasis patients. Deficiency of another enzyme also causes hyperhomocysteinemia is

MTHFR. Mutation or polymorphism may contribute this type of declination in enzyme activity which may hamper methionine and homocysteine metabolism and may lead to hyperhomocysteinemia or hyperhomocysteinuria. Association between MTHFR gene polymorphism and psoriasis has attracted great interest amongst researchers worldwide. The MTHFR gene (NM_005957) is located at chromosome 1 (1p36.3). It catalyses the conversion of 5,10 methylenetetrahydrofolate to 5-methylenetetra-hydrofolate, which then leads to the remethylation of homocysteine to methionine [Liew et al 2012]. This cycle is important for maintaining the methyl donors for DNA methylation, and hence gene regulation and cellular differentiation [Liew et al 2012]. Polymorphism of the MTHFR gene involves the substitution of the nucleotide C with T at position 677 which leads to transition of Alanine to Valine. This reduces MTHFR enzymatic activity and thermostability, therefore interferes with the homocysteine levels [Enbersen et al. 2012, Liew et al 2012, Frosst et al. 1995, Jacques et al. 1996, Santosh et al 2010]. High plasma homocysteine has been found in most of the psoriasis patient studied in our present work. Plasma homocysteine increases with age and one of the major factor contributing to increase plasma and urinary homocysteine is declination of renal function with age. Our patients also showed urinary incontinence with hyperhomocysteinuria. Homocysteinemia in our population is characterized by relatively moderate to high increase in plasma homocysteine (56 µmol/L) where the urinary excretion of homocysteine is 29 µmol / mmol of creatinine which is also signifies hyperhomocysteinuria. In addition to this, plasma cysteine level of 211 µmol/L in normal and 201 µmol/L in psoriasis which are found to be within the normal range. Severe hyperhomocysteinuria is always coincide with a relatively low cysteine level in plasma and urine but in our patient population the cysteine level remained more or less normal which indicate anon severe type of homocysteinuria in psoriasis.

Traditionally hyperhomocysteinemia and hyperhomocysteineuria commonly found in people with vit B6, B9 (folic acid) and B12 deficiency. Other causes of hyperhomocysteineuria are genetic defects [Pal & Arora et al. 2012, Engberson et al. 1995, Beard et al 2011, Ignoul et al 2005].

MTHFR catalyse the conversion of5,10 methylene tetrahydro folate to 5, methylene tetrahydro folate to facilitate the further conversion to methionine. This methionine donates its methyl group to methyl acceptor and transform to Adenosyl homocysteine. Adenosyl homocysteine then form the cellular homocysteine pool (fig 1). Therefore any alteration in the methionine pool of MTHFR enzyme dependent pathway may alter the cellular homocysteine metabolism and give the altered pool for homocysteine which then generate homocysteinemia and homocysteineuria [Christensen et al. 1999, Shield et al.1999, Jacques et al. 1996]. Vitamin B12 deficiency has been positively associated with hyperhomocysteinemia in a group of patients with general neurological deficiencies, vascular disease, Psychomotor disturbances and cognitive failure and recurrent pregnancy loss [Fenech et al. 1997, Govindaiah et al. 2000, Van der Put et al. 1998, Varga et al. 2005]. Severe hyperhomocysteinemia has been reported to cause neurological abnormalities, mental retardation, arteriosclerosis, and thrombosis [Engbersen et al. 1995, Van der Put et al 1998, Isotalo et al. 2000].

Contradictory reports were also available on MTHFR mutation in psoriasis vulgaris indicated no significant association of MTHFR mutation with psoriasis and there was no evidence of hyper homocysteinemia [Liew et al.2012]. In our present work we did not studied the polymorphic status of MTHFR gene of psoriasis patients and control population. But a significant increase in plasma and urinary homocysteine level was observed. The activity of the enzyme MTHFR, CβS and MS was not studied. But we observed urinary incontinence in patience with homocysteinemia and homocysteineuria in our psoriatic patient population.

Homocyateine is a potent excitatory neurotransmitter that binds with NMDA receptor and leads to oxidative stress, calcium influx in cytoplasm, cellular apoptosis and resulting endothelial dysfunction. Depletion of cellular ATP reserve is one of the major causes of decreased cellular respiration and cell death [McCully et al.2009]. Homocysteine by regulation the calcium influx in the cytoplasm alter the excitatory signal generation and propagation in neuronal cell and thereby responsible for generating neuronal excitotoxicity. Increased homocysteine level in CSF has been found to cause neuronal necrosis [Olney et al. 1974]. In psoriasis also increased level of plasma and urine homocysteine causes endothelial dysfunction [Pal & Arora 2012] in the vascular bed leading to circulatory abnormality which may also affect the kidney vascular bed. Neuronal excitotoxicity may produce neuronal dysfunctioning in the bladder and produces urinary incontinence. Increased homocysteine level in the blood and tissue may induce oxidative damage which may also cause neuronal dysfunctioning with cellular death. S-adenosyl homocysteine which is also found to be higher in our patient group may have some relation with the urinary incontinence. S- adenosyl Homocysteine is a non competitive inhibitor [Zhu et al. 2002] of catechol oxy methyl transferase (COMT) the enzyme responsible for catecholamine reuptake by methylation of the neurotransmitter in the presynaptic terminal. Deficiency of the COMT leads to build up of catecholamines in the nerve terminals and in blood and tissue space. This increased tissue catecholamine pool may contribute to the malfunctioning of neuronal tissue with increased reactive oxygen species leading to abnormalities in the functional homeostasis. Psoriasis is also known to cause differential expression of nerve growth factors and neurotropins

[Truzzi et al. 2011] which may also give rise to reduction in nerve conduction velocity and may be a potential cause of neuro degeneration with peripheral and vascular neuropathy.

However, psoriasis with urinary inconsistency can be judged in the light of metabolic error with hyperhomocysteinemia and hyperhomocysteinuria with critical outlook of homocysteine and methyionine imbalance. Here, hyperhomocysteinuria can be used as a prognostic marker for psoriasis.

Further intensive research should be done to conclude the involvement of different mutations in the enzymes involved in transmethylation/ transsulferation pathway with relative activity of those enzymes to correlate with the reaction grade of transmethylation / transsulfuration pathway. Plasma and urinary amino acid concentration should be correlated with the light of enzyme activity and mutational spectra of the genes involved in this metabolic pathway. The urinary incontinence reflected by psoriasis patients should be studied with greater responsibility. Another is to find out the histopathological and electrophysiological changes in urinary bladder with psoriasis and abnormal homocysteine level in plasma and urine and its correlation with urinary incontinence. Nerve conduction velocity measurement is another way to find out the effect of high homocysteine exposure in persons with urinary incontinence. Clinicians can try vitamin B12 and folic acid loading to treat urinary incontinence with most conscious dose selection to find out the remedy.

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REFERENCES

[1] Beard RS, Bearden SE. *Am J Physiol*. **2011**.300: H13-26.
[2] Bowcock AM, Barker JN. *J.Am. Acad. Dermatol*.**2003**. 49(2 suppl.): S51-56.
[3] Brazelli V, Grasso V, Fornara L. Et al. *Int. J. Immunopathol. Pharmacol*. **2010**. 23(3): 911-6.
[4] Cakmak SK, Giil U, Kilic C. et al. *J. Eur. Acal. Dermatol. Venereal*. **2009**. 23(3): 300-303.
[5] Christensen B, Arbour I, Tran P et al. *Am. J. Med. Genet*. **1999**. 84: 151-7.
[6] Fenech MF, Dreosti IE, Rinaldi JR. *Carcinogenesis*.**1997.** 18: 1329-36.
[7] Frosst P, Blo H, Milos R, Goyette P et al. *Nat Genet*. **1995**. 10:111-3.
[8] Engbersen AMT,Franken DG, Boers GHJ et al. *Am.J. Hum. Genet*.**1995**. 56:142-150.
[9] Govindaiah V, Naushad SM, Krishna PC, Devi ARR. *Clinical. Biochemistry*. **2000**.42:382-6.
[10] Ignoul S, Eggermont J.: *Am.J. Physol.Cell Physiol.* **2005**. 289: C1369-78.
[11] Isotalo PA, Donnelly JG. *Mol. Diagn***. 2000**. 5: 59-66.
[12] Jacques P, Bostom A, Williams R et al. *Circulation* **1996.** 93:7-9.
[13] Krishnamoorthy G. And Mohan V. *Obstetrics and Gynecology*. **2012**. 1-22.
[14] Liew SC, Dasgupta E, Wong SF et al. *Nutrition* J. **2012**. 11: 1-7.
[15] Malbera M, Gisondi P, Radaeli A. Et al. *Br.J. Dermatol*. **2006**. 155 (6): 1165-9.
[16] Mc Cully KS. *Ann. Clin. Lab Sci.* **2009.** 39: 219-32.
[17] Olney JW. Toxic effects of Glutamate and related aminoacids on the developing central nervous system. In: Heritable disorders of Amino Acids Metabolism Nyhan WL, ed, New York: John Wiley and Sons. **1974**. 501-12.
[18] Paige DG. Skin Disease. In: Clinical Medicine(vii th Edi), Edi: Kumar P. Clark M. Sounders, Elsevier.pp1240-42.
[19] Pal N. & Arora RS. *Obstratics and Gynecology*. Elsevier, **2012**. 23-46.
[20] Santos M, Silva F, Gomes K et al. *Mol Biol Rep.* **2010**. 38:3361-6.
[21] Schofield OMV, Rees JL. Skin Disease. In: Davidson's Principles and Practice of Medicine (21st Edi), Edi: Colledge NR, Walkes BR, Ralston SH. Pp 1237-40.
[22] Shield DC, Kirke PN, Mills JL et al. *Am. J. Hum. Genet***. 1999.** 64: 1045-55.
[23] Truzzi F, Marconi A, Pincelli C. *Dermas-Endocrinology* .**2011.** 3:1, 32-36.
[24] Tsoi LC, Spain SL, Knight J et al. *Nat Genet.* **2012**. 44(12):1341-8. doi: 10.1038/ng.2467.
[25] Varga EA, Sturm AC, Misita CP, Moll S. *Circulation*. **2005.** 111: e289-93.
[26] Van der Put NMJ, Gabreels F, Stevens EMB et al. *Am. J. Hum. Genet*. **1998**.62: 1044-51.
[27] Zhu BT. *Histol and Histopathol*. **2002.** 17: 1283-91.