India is the “diabetes capital of the world”
India’s Diabetes Boom

Nature 2012;485:S14–S16

Diabetic retinopathy (DR)

Diabetic retinopathy occurs in 87.5% of all persons having diabetes for >15 years.

The severity of DR proportionately increased with longer duration of diabetes.
Diabetic retinopathy

Inside picture....

Retinal vascular microaneurysms, blot hemorrhages, cotton-wool spots, loss of retinal pericytes, increased vascular retinal permeability, alterations in regional blood flow, and abnormal retinal microvasculature, retinal hemorrhage

Vision loss

84% Indian population suffering from hyperhomocysteinemia

Hyperhomocysteinemia is an independent risk factor in diabetic retinopathy
Hyperhomocysteinemia

Elevated levels of Homocysteine concentration in blood is known as Hyperhomocysteinemia

Cysteine

↑

Pyridoxal 5’-phosphate & Methylcobalamin

Homocysteine

↓

L-methylfolate

Methionine

MTHFR

Folic acid

Deficiency of L-methylfolate, Pyridoxal 5’-phosphate is the predominant cause of hyperhomocysteinemia

MTHFR: methylenetetrahydrofolate reductase
Hyperhomocysteinemia is associated with Retinal Ganglionic Cell loss seen in Indian population.
Hyperhomocysteinemia

Neuronal death  Retinal vein occlusion  VEGF mRNA expression  Oxidative stress

Retinopathy

Elevated homocysteine increased steady state VEGF mRNA levels 4.4-fold

Diabetic retinopathy
Plasma and vitreous homocysteine concentrations in patients with proliferative diabetic retinopathy

- 20 patients with PDR and 12 nondiabetic patients with nonproliferative ocular diseases

- Plasma and vitreous samples were obtained to measure

Vitreous Hcy concentrations were elevated in patients with PDR probably due to breakdown of the blood-retina barrier.

Homocysteine concentration was 74% & 29% higher in plasma and vitrous in PDR than control patients.

*P<0.001

Plasma, aqueous and vitreous homocysteine levels in proliferative diabetic retinopathy (PDR)

- 20 eyes with PDR and 21 eyes of patients without diabetes mellitus were examined

- Blood plasma, aqueous and vitreous samples were collected during combined cataract and pars plana vitrectomy for homocysteine measurement
Homocysteine concentration was 30% higher in PDR than control patients.
A cross-sectional case-control study

100 normal control subjects and 300 subjects with type-2 diabetes (T2D).

Of the 300 subjects with T2DM, 200 had diabetic retinopathy (DR) and 100 did not (DNR).
% Prevalence of hyperhomocysteinemia with >12µmol/L

- Controls: 12%
- DNR: 48%
- DR: 64%

*P<0.05
Contd..

Homocysteine concentration

- Controls: 7.8 µmol/L
- DNR: 12.8 µmol/L
- DR: 15.3 µmol/L

*P<0.05
Folic acid deficiency

**Controls**

Folic acid conc (ng/ml): 10

**DNR**

Folic acid conc (ng/ml): 7.8

**DR**

Folic acid conc (ng/ml): 7.2

*P<0.05

Pyridoxine deficiency

- Controls: 20.6 ng/ml
- DNR: 13 ng/ml
- DR: 14.6 ng/ml

*P<0.05

Plasma vitamin B12 deficiency

Controls: 385 ng/ml
DNR: 272 ng/ml
DR: 144 ng/ml

P<0.05
• Plasma total homocysteine was measured in 56 consecutive patients with recently diagnosed retinal vascular occlusive disease:

• 36 had central retinal vein occlusion, 12 branch retinal vein occlusion, and 8 retinal artery occlusion, and compared them with 59 age- and sex-matched healthy controls.
Homocysteine concentration

- Control: 8.96 µmoles/l
- Retinal vein occlusion: 15.3 µmoles/l
- Retinal artery occlusion: 20.95 µmoles/l

*P<0.001

Each 1 μmol/l increase in homocysteine was associated with a 7% increased odds of RVO.

**Conclusion**

The data indicate that hyperhomocysteinemia & deficiency of B-vitamin could be an independent risk factor for DR.

Regardless of dietary intake of B-vitamins, MTHFR Polymorphism is a risk factor for Diabetic Retinopathy
MTHFR Polymorphism leads to deficiency of active L-methylfolate concentration...causing Hyperhomocysteinemia
Prevalence of MTHFR Polymorphism

60%

Of the total population are having MTHFR genetic polymorphism
There are two types of MTHFR genotypes, TT & CC

MTHFR C allele is physiologically protective and T allele is responsible for increased metabolic risk in Indian population.

Father

Mother

TT

CC

TT

CT

TT

CT

CC
MTHFR enzyme activity is reduced by 35% among the 677CT carriers and by 50% to 70% among 677TT carriers.
MTHFR Polymorphism in Uttar Pradesh

MTHFR Polymorphism is predominant in Uttar Pradesh

BHU Varanasi, March 2012 Report

Ind J Hum Gen Jan 2012 Report

BHU Varanasi, Feb 2012 Report

South Indian Study 2004

Homozygosity (TT) and heterozygosity (CT) for the MTHFR polymorphism

MTHFR polymorphisms was found to be predominant among Tamilians
MTHFR polymorphisms was prevalent among Bramhin & Rajputs of Uttar Pradesh

Eastern Uttar Pradesh Report 2010

High MTHFR Polymorphism in Muslim population
The relationship between MTHFR gene polymorphisms, plasma homocysteine levels and diabetic retinopathy in type 2 diabetes mellitus

Total of 208 patients with type 2 diabetes mellitus and 57 controls were recruited into the study.

MTHFR polymorphism is strongly associated with hyperhomocysteinemia and diabetic retinopathy
MTHFR polymorphism & DR

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>DNR</th>
<th>DR</th>
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<tbody>
<tr>
<td>MTHFR TT</td>
<td>17.54</td>
<td>29.59</td>
<td>28.18</td>
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<tr>
<td>MTHFR CC</td>
<td>28.07</td>
<td>29.59</td>
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<td>Allele T</td>
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An updated meta-analysis of methylenetetrahydrofolate reductase gene 677C/T polymorphism with diabetic nephropathy and diabetic retinopathy
An updated meta-analysis of methylenetetrahydrofolate reductase gene 677C/T polymorphism with diabetic nephropathy and diabetic retinopathy

ABSTRACT

Studies investigating the association of methylenetetrahydrofolate reductase (MTHFR) gene 677C/T polymorphism with diabetic nephropathy and diabetic retinopathy have so far reported inconclusive results. We therefore aim to address this inconclusiveness by conducting a meta-analysis. Random-effects model was applied irrespective of between-study heterogeneity. Data and study quality were assessed in duplicate. A total of 7807 and 1599 subjects from 21 and 8 studies were analyzed for diabetic nephropathy and diabetic retinopathy, respectively. Carriers of 677TT genotype were 1.71 (95% confidence interval [95% CI]: 1.02–2.88; \( P = 0.042 \)) and 2.89 (95% CI: 1.51–5.53; \( P = 0.001 \)) times more likely to develop diabetic nephropathy separately relative to diabetic patients without nephropathy and nondiabetic controls. Likewise, this association was preserved for diabetic patients with retinopathy referring to those without (odds ratio [OR] = 1.86; 95% CI: 1.21–2.86; \( P = 0.004 \)). Subgroup analyses showed that ethnicity was a possible confounder, especially in West Asians and Africans, and so were gender and duration of diabetes mellitus in diabetic nephropathy studies. Probability of publication bias was low across all comparisons as reflected by the funnel plot and corresponding test. Taken together, our results demonstrate that MTHFR gene 677TT genotype might confer a moderately augmented risk for diabetic nephropathy and diabetic retinopathy.
...hence this arises the need of

Active supplementations of conventionally used vitamins...
Introducing

**Foliact-N**

L-methylfolate 1mg + Methylcobalamin 1500mcg + Pyridoxal 5-phosphate 10mg
In a randomized, placebo-controlled, double-blind trial, 35 patients with endothelial dysfunction were randomized to Combination of LMF + P5P + Methylcobalamin or placebo for 8 weeks.
Triple combination significantly improved endothelial function by 136% at 8 weeks.

Baseline: 3.6%
After 8 weeks: 8.5%
P = 0.021

FMD: flow mediated dialation

Arterioscler Thromb Vasc Biol. 2006; 26: e43-e52
Increasing retinal blood flow

Improving retinal function
Conventional formulations

- Folic acid
- Vitamin B6
- Vitamin B12

Inactive

Active

- L-methylfolate
- Pyridoxal 5’-Phosphate
- Methylcobalamin
Bypasses MTHFR polymorphism

Homocysteine

↓

L-methylfolate

Methionine

Active L-methylfolate.....decreases homocysteine levels
L-methylfolate vs folic acid

Homocysteine reduction after 24 weeks

Placebo  Folic acid  LMF

% Hcy reduction

-16  -14  -12  -10  -8  -6  -4  -2  0  2  4  6  8  10  12  14  16

-14.6  -9.3  0

P<0.05

**L-methylfolate vs folic acid**

- L-methylfolate: -20%
- Folic acid: -7%

3 times greater homocysteine reduction than folic acid

College of Medicine, Univ of South Alabama, submitted for Publication, data on file
L-methylfolate vs folic acid

Cmax

Cmax (ng/ml)

129

L-Methyl folate

9 times more concentration than conventional folic acid

14.1

Folic acid

British Journal of Pharmacology 2004;141:825–830
**L-methylfolate vs folic acid**

Tmax

- **Folic acid**: 2.3
- **L-methylfolate**: 1.3

60 min earlier onset of action than folic acid

British Journal of Pharmacology 2004;141:825–830
L-methylfolate vs folic acid

AUC

L-Methyl folate: 383
Folic acid: 73

5 times more bioavailable than conventional folic acid

British Journal of Pharmacology 2004;141:825–830
Conventional Preparation Folic Acid Vitamin B-6

Activation Steps

High T-max
Less Bioavailability
Less Cmax
Patients metabolism disorder

Results in less reduction in Hyperhomocysteinemia

Active metabolites for Folic Acid, Vit-6, 12 Are Essential

P5P & Methylcobalamin

L-methylfolate
L-methylfolate

Pyridoxal 5’-Phosphate

Methylcobalamin

More Bioavailability & Cmax
Low dose of Vit.

More reduction in HHcy

Low T-max
Faster Absorption
Indication & dosage

• For the prevention of diabetic retinopathy, venous occlusion

• One tablet OD