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# TREATMENT WITH ORAL METAFOL L APPEARS TO PROMOTE RESTORATION OF LOST CUTANEOUS SENSATION IN DIABETIC PERIPHERAL NEUROPATHY

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**ABSTRACT:** The purpose of this research work is to determine whether Metafol-L improves sensory neuropathy in patient with Type 2 diabetes or not. In patients with diabetes mellitus, prolonged hyperglycemia can result in peripheral neuropathy (pain and/or loss of sensation in the extremities). Neuropathy issues may be related to Vitamin B deficiencies; therefore, the supplementation of Vitamin B12, Vitamin B6, and folate (Vitamin B9) may improve diabetic peripheral neuropathy (DPN). Metafol L is a product containing L-methyl folate, pyridoxal 5 phosphate, and methylcobalamine for management of endothelial dysfunction. Metafol-L ingredients counteract endothelial nitric oxide synthase uncoupling and oxidative stress in vascular endothelium and peripheral nerve. Studies of monotherapy with l-methylfolate, methylcobalamine and pyridoxine 5 phosphate suggest that each of these bioavailable B vitamins may reverse the pathophysiology and symptoms of DPN. After approximately six month of treatment, patient undergoes follow up biopsy. At the end of their treatment 79% of patient showed an increase in calf ENFD & 87% of patient show experienced both reduced frequency and integrity of paraethesias and or dysesthesias.

**INTRODUCTION:** Diabetic neuropathy is nerve damage caused by diabetes. High blood sugar can reduce blood flow and injury nerve fibers throughout your body, but most often the damage occurs in your legs and feet. The prevalence of diabetes mellitus (DM) in Indian population was about 7.8% in 2015 [1-3]. Up to 60% of DM patients have diabetic peripheral neuropathy (DPN), a major cause of disability in the India. DPN is primarily due to deterioration in small nerve fibers (myelinated A-" and unmyelinated C-fibers) that mediate pain, temperature, and autonomic functions.[4]

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At least 6 out of 10 people with diabetes have solved degree of neuropathy making it the most common complication of diabetes. [1-4] Diabetes neuropathy with loss of feeling in your feel is one of the most frequent causes of feat ulceration and amputation. Diabetic neuropathy is progressive disease, it develops generally and you may not notice until the damage has already occurred. You can prevent diabetic neuropathy or slow it process with tight sugar control and healthy lifestyle. [1-5]

- Mild/moderate pain burning, shoaling, stabbing pains
- Severe pain (sever burning, shooting and stabbing pains, increased sensitivity)
- Sensory loss.

- Numb less/ deadness
- Painful injury
- Redness thermal sensation

- Late complication

- Ulceration, foot deformility, non traumatic amputation.

Cause of Diabetic Neuropathy is elevated blood sugar level, damage the blood venal, and reduce blood flow to nerves, storming them of the oxygen and nutrients they need to survive. Elevated blood sugar directly damages the nerves resulting in reduced nerves fiber density. Currently, agents used to reduce symptoms of pain and painful dysesthesias of SFN include anticonvulsants, tricyclic antidepressants, selective serotonin reuptake inhibitors, serotoninnorepinephrine reuptake inhibitors, and opioid or opioid-like analgesics.

Among these medications, only duloxetine and pregabalin have been approved by the US Food and Drug Administration (FDA) for treatment of these symptoms. Unfortunately, there is no evidence that any of these agents modify the underlying pathophysiology of SFN. Thus, these medications exhibit merely palliative activity for SFN. Clearly, there is a need for agents that modify the underlying pathophysiology of DPN and, thus, SFN. [11] Metafol L the active, naturally occurring form of it B6, B12 and folic acid. Up to 50% of individual are unable to fully convert folic acid into active form of foliate: L-methylfolate: [1-3].

Metafol-L is medicated food available by prescription designed to nutritionally manage the metabolic process that regulate blood vessels & nerve health in patient with diabetic neuropathy. Another little known fact is that third party lab testing on finished product is not required by FDA or CGMP process. However, at Metafol L we feel this is extremely important as it allow us to validate the work of our contract manufactured and it's just another check we can do to ensure our product are made to standard we require for our customer.

A little known fact many companies are using generic methylfolate ingredient that are amorphous salt based (even some patented ingredients are amorphous salt based). These amorphous salt based ingredients can lose up to 16% potency per year because they are less stable. And if these companies don't formulate with very large amount of overage in the formula, the product will not be as potent as claim on the lable says it is. However, metafol l uses internationally patented crystalline salt based methylfolate, which should not lose more than 2% potency per year due to its crystalline nature.

Improvement in epidermal nerve fiber density (ENFD), assessed using the skin punch biopsy method developed by Polydefkis and colleagues, [3] is a surrogate marker of improvement of DPN or, more narrowly, SFN. This highly sensitive and reliable method of measuring ENFD to assess severity of SFN and response to treatment was an ideal technique for this study. The skin punch biopsy method, which involves direct quantification of pathologic changes in epidermal nerve fibers, is useful in identifying SFN due to various causes, including impaired glucose tolerance. [17-19]

We hypothesized that an orally administered combination of L-methylfolate, methylcobalamin, and pyridoxal 5!-phosphate (LMFMC- PP) improves ENFD and, thus, SFN, in symptomatic type 2 DM patients with established SFN. The current clinical investigation was a case series of SFN patients in which immunohistochemical analysis of specimens obtained via skin punch biopsy was used to evaluate SFN and its response to treatment.

## PATIENTS AND METHODS:

**Patients:** Sixteen consecutive patients with confirmed type 2 DM, as well as symptoms consistent with SFN of the feet, were recruited from the Diabetologist Dr. Pallavi Shende. Verbal consent was obtained from each patient after the study, procedure, and possible complications were described in detail. The study was conducted between Dec 2015 and Sept 2016.

Inclusion criteria consisted of diabetes with a history of both positive and negative sensory symptoms (*eg*. paresthesias, spontaneous pain, or dysesthesias) of the lower extremities. Exclusion criteria included history of hereditary neuropathy, use of any medication for diabetic neuropathy, and presence of any type of peripheral neuropathy that was not due to diabetes.

Information regarding symptoms, duration of diabetes, current medications, and com orbid conditions was obtained from each patient. Each patient reported symptoms consistent with SFN, such as numbness, burning, tingling, cramping, or weakness of the lower extremities. Each patient rated these symptoms at both onset and completion of treatment using a visual analog scale (VAS).

Also, patients underwent a full baseline physical examination that included assessment of sensitivity to vibration, light touch, and cold temperature, as well as response to 10-g monofilament.

**Methods:** Consecutive type 2 DM patients with symptomatic DPN were assessed for ENFD at the calf by means of skin punch biopsy and then placed on once daily oral-combination L-methylfolate (3 mg), methylcobalamine (2 mg), and pyridoxal 5!-phosphate (35 mg) for approximately 6 months. Patients then underwent follow-up biopsy **(Table 1**).

The standard biopsy protocol involved sterilizing the biopsy site with povidone-iodine and anesthetizing the site with 3 mL of injectable 0.5% bupivacaine hydrochloride with epinephrine 1:200,000. During the biopsy procedure, two 3-mm cutaneous punch biopsies (separated by a distance of about 5 mm) were obtained from a site 10 cm proximal to the lateral malleolus between the peroneal and Achilles tendons at a calf of each patient. Specimens were preserved in a standard fixative solution consisting of 2% periodate lysine paraformaldehyde and then sent for histologic analysis to NCORD (Pune, India).

The method of ENFD evaluation relied on immunohistochemical localization of a neural antigen within axons. Tissue sections from the 3-mm punch biopsy specimens were cut to 50 #m in thickness and stained with polyclonal antibodies recognizing the protein gene product (PGP) 9.5, which is present in all nerve fibers in the skin. To obtain the ENFD in fibers per millimeter (fibers/mm) of epidermis, pathologists manually counted the number of epidermal nerve fibers in three to five sections and divided the value by the sum of the lengths of the epidermal specimens in millimeters.[20] Pathologists were blinded to whether biopsy specimens were obtained before or after treatment. The primary efficacy endpoint was improvement in ENFD after approximately 6 months of treatment.

**Statistical Analysis**: Statistical analysis was conducted by using SAS® 9.2 (SAS Software, Pune). The research hypothesis was that LMF-MCPP increases ENFD among study patients with SFN. A signed-rank test was used to determine whether, for the average patient, ENFD increased significantly after 6 months of treatment with LMF-MC-PP. Spearman correlation based on rank was used to determine whether a difference in fiber density was related to age, sex, or duration of diabetes. Results were expressed in mean change in ENFD from baseline over the 6-month interval, as well as *P* value. In this study, a *P* value less than 0.05 was considered statistically significant.

**RESULT:** Among the 16 patients (8 men, 8 women, aged 29-78 years), duration of type 2 DM ranged from % 6 months to & 20 years (**Table 1**). At baseline, each of the 16 patients underwent two ENFD punch biopsies and was then placed on one oral tablet of combination LMF-MC-PP bid for approximately 6 months (median 5.7 ' 0.6 months). Although two post-treatment punch biopsies were obtained from patients. Oral-combination LMF-MC-PP was well tolerated and no patient reported any adverse events. There were no adverse events due to biopsy.

**Change in ENFD and Symptoms after 6 Months of Treatment:** ENFD was assessed by immunohistochemical staining of biopsy samples. The values for average baseline, post treatment, and increase in ENFD in fibers/mm for each of the 16 patients are shown in **Table 2** and illustrated in **Fig. 1**. The mean ENFD of the 16 participants was 1.83 fibers/mm at baseline and 3.93 fibers/mm after approximately 6 months of oral treatment with combination LMFMC- PP, representing a 97% increase in ENFD (*P* (.004) (**Fig. 2**). Fourteen of the 16 (79%) patients experienced an increase in ENFD during an approximately 6-month course of treatment with LMF-MC-PP (**Fig. 1**). The mean per-patient increase in ENFD was 1.5 fibers/mm. Comparison of immunohistochemically stained sections of epidermis revealed improvements after approximately 6 months of treatment with combination LMF-MC-PP.

The photomicrographs in **Fig. 3** show immunohistochemically stained baseline and post-treatment sections of epidermis from patient 12. The post-treatment section shows regenerating small nerve fibers in both the basement membrane and keratin layers. Based on ENFD measurement, the patient experienced a mean increase of 3.93 fibers/mm during approximately 6 months of treatment. A total of 87% of study patients reported reduced frequency and intensity of paresthesias and dysesthesias after 6 months of treatment.

**Statistical Analysis of Changes in ENFD after Treatment:** For the changes in ENFD, the Wilcoxon test statistic was 22.5, with a derived *P* value of 0.004, which is much smaller than a conventional type I error rate of 0.05. This finding indicated that, for the average patient in the study, the increase in ENFD after approximately 6 months of treatment with oral-combination Metafol L was statistically significant. The Spearman correlations were 0.30 (*P* (0.40) between increase in ENFD and age;) 0.36 (*P* ( .28) between increase in ENFD and sex, favoring female sex (though not significantly); and 0.75 (*P* ( 0.02) between increase in ENFD and duration of diabetic history. Thus, only duration of diabetes correlated significantly with increase in ENFD after therapy with LMF-MC-PP, implying that a longer duration of diabetes might be associated with a greater increase in ENFD after treatment.

**TABLE 1: PATIENT CHARACTERISTIC**

| Patient | Age of patient | Sex | Duration of diabetes mellitus (y) |
|---|---|---|---|
| 1 | 72 | M | 2 |
| 2 | 56 | M | 7 |
| 3 | 54 | M | 8 |
| 4 | 47 | F | 10 |
| 5 | 29 | M | 3 |
| 6 | 65 | M | 7 |
| 7 | 45 | F | 5 |
| 8 | 78 | F | 20+ |
| 9 | 62 | M | 10 |
| 10 | 51 | F | 5 |
| 11 | 39 | F | 0.5 |
| 12 | 46 | M | 8 |
| 13 | 58 | M | 3 |
| 14 | 55 | F | 10 |
| 15 | 71 | F | 20+ |
| 16 | 56 | F | 7 |

**TABLE 2: DURATION OF TREATMENT AND AVERAGE BASELINE, POST TREATMENT AND INCREASE IN ENFD VALUE**

| Patient | Duration of treatment (Mo) | Average baseline ENFD (Fibers/mm) | Average post treatment ENFD (fibers/mm) | Average increase in ENFD (Fibers/mm) |
|---|---|---|---|---|
| 1 | 5.2 | 0.17 | 3.60 | 3.43 |
| 2 | 5.7 | 1.28 | 4.5 | 3.22 |
| 3 | 6.0 | 2.56 | 6.98 | 4.42 |
| 4 | 5.5 | 0.65 | 2.5 | 1.85 |
| 5 | 5.9 | 3.45 | 4.32 | 0.87 |
| 6 | 5.5 | 4.19 | 4.65 | 0.46 |
| 7 | 6 | 1.23 | 3.98 | 2.75 |
| 8 | 5.8 | 0 | 4.12 | 4.12 |
| 9 | 5.2 | 4.17 | 5.12 | 0.95 |
| 10 | 5.8 | 0 | 0 | 0 |
| 11 | 5.5 | 2.44 | 4.87 | 2.43 |
| 12 | 6.0 | 1.12 | 7.10 | 5.98 |
| 13 | 6.1 | 0.89 | 3.21 | 2.32 |
| 14 | 5.1 | 3.99 | 6.98 | 2.99 |
| 15 | 5.7 | 0 | 0 | 0 |
| 16 | 5.8 | 3.15 | 6.12 | 2.97 |

**ENFD: Epidermal nerve fiber density:**

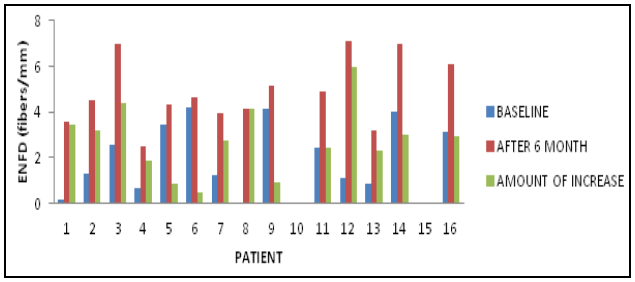


**FIG. 1: EIGHT OF THE 16 PATIENTS (79%) EXPERIENCED AN INCREASE IN ENFD DURING AN APPROXIMATELY 6-MONTH COURSE OF TREATMENT WITH THE ORAL COMBINATION METAFOL L. FOR PATIENT 8, THE BASELINE ENFD VALUE WAS ZERO. FOR PATIENTS 10 AND 15, BOTH BASELINE AND POST-TREATMENT ENFD VALUES WERE ZERO**


**FIG. 3: MEAN ENFD OF 16 PATIENTS WAS 1.83 FIBERS/MM AT BASELINE AND 3.93 FIBERS/MM AFTER 6 MONTH OF TREATMENT**

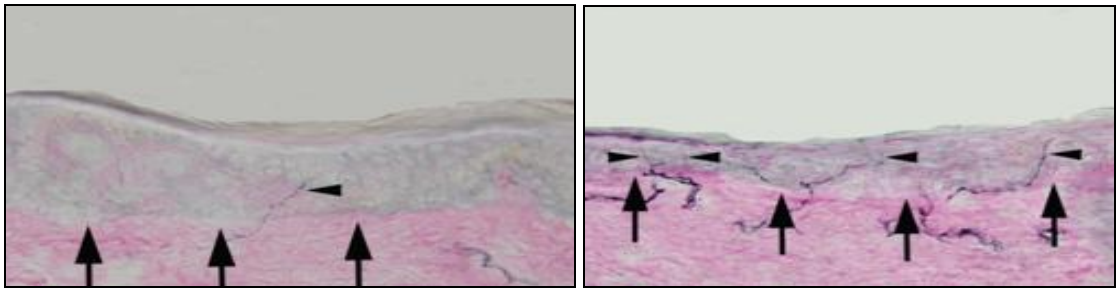

**FIG. 3: PHOTOMICROGRAPHS OF IMMUNOHISTOCHEMICALLY STAINED SECTIONS OF EPIDERMIS FROM THE LEFT CALF OF PATIENT 12, WHO HAD TYPE 2 DIABETES AND DIABETIC SMALL-FIBER NEUROPATHY AND WAS TREATED WITH METAFOL L ONCE DAILY FOR 6 MONTHS. (A) PHOTOMICROGRAPH OF BASELINE SKIN PUNCH BIOPSY TAKEN FROM THE LEFT CALF REVEALED LOW ENFD. THE BASEMENT MEMBRANE IS INDICATED BY THREE VERTICAL ARROWS. AN INTRAEPIDERMAL NERVE FIBER IN THE KERATIN LAYER IS INDICATED BY A HORIZONTAL TRIANGLE. (B) PHOTOMICROGRAPH OF A SIMILAR SAMPLE FROM THE SAME PATIENT TAKEN AFTER APPROXIMATELY 6 MONTHS OF TREATMENT. BASED ON MEASUREMENT OF ENFD, A MEAN INCREASE OF 3.93 NERVE FIBERS/MM HAD OCCURRED DURING TREATMENT. REGENERATING SMALL NERVE FIBERS CAN BE SEEN IN BOTH THE BASEMENT MEMBRANE (INDICATED BY FOUR VERTICAL ARROWS) AND KERATIN LAYER (INDICATED BY HORIZONTAL TRIANGLES)**

## DISCUSSION:

**Potential Benefits of Metafol L Therapy in Patients with SFN:** This is the first clinical study to suggest that treatment with Metafol L may promote statistically significant improvement in ENFD in patients with SFN. The finding that this improvement in ENFD was associated with decreased anesthesia, paresthesia, or dysesthesia in greater than 80% of study patients implies that improvement in these symptoms was due to increased ENFD. These results were consistent with those of a 2009 pilot study by Walker and colleagues 21 in which a series of type 2 diabetic patients with SFN who received LMF-MC-PP for 1 year experienced progressive and statistically

significant improvement in cutaneous sensitivity of the feet, as measured by one- and two-point static Pressure-Specified Sensory Device testing. Together, these two studies provide evidence that LMF-MC-PP promotes restoration of damaged cutaneous nerve fibers in patients with SFN.

The findings of the current study also are consistent with the theory that treatment with medical nerve foods that promote the bioavailability of endothelial NO may alter the underlying pathogenesis of DPN and consequently SFN. As noted earlier, evidence indicates that each of thecomponents of LMF-MC-PP may have the potential to improve SFN by modifying its underlying pathophysiology. Both pyridoxal 5!-phosphate and methylcobalamine monotherapy may be essential to critical peripheral nerve functions that are impaired in DPN. [14, 15, 22] L-methylfolate has been shown to improve endothelial function in patients with type 2 DM, possibly by promoting synthesis of NO.[12, 13, 23]

**CONCLUSION:** This study suggests that oral administration of Metafol L promotes increase in ENFD in participants with diabetic SFN and improves symptoms of anesthesia, paresthesia, or dysesthesia. A high-quality, doubleblinded, randomized, controlled trial is necessary to confirm the effectiveness of LMF-MC-PP in SFN. Meanwhile, LMF-MC-PP should be considered in the treatment of patients with diabetic SFN in whom other therapies have been ineffective.

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