The sensory disturbances in patients stroke combined with small fiber neuropathy

Xiaojing Liu[1], Aiqin Liu[1], Xinjing Liu[1], Jing Yang[1], Changhe Shi[1], Jun Wu[1], Yuming Xu[1], Baohan Pan[2].

[1] Department of Neurology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China
[2] Johns Hopkins School of Medicine, Baltimore, MD, USA.
Emails: xiaojing1022@sina.com, xuyuming@zzu.edu.cn

Abstract: Objective: To study the combined sensory impairment in stroke patients in skin around the nerve fiber damage. **Methods**: 20 cases stroke patients and 20 healthy controls of age and sex matched were collected. Skin biopsy was performed and skin taken to observe the morphology of small nerve fibers. Epidermal nerve fiber density declined in stroke group and negatively correlated with age, duration. Comparison between two groups yielded non discriminatory nerve fiber density difference as well as no difference in gender, age within each group was seen. Conclusion: stroke patients with combined sensory disorders have epidermal nerve fiber damage.
[Xiaojing Liu, Aiqin Liu, Xinjing Liu, Jing Yang, Changhe Shi, Jun Wu, Yuming Xu, Baohan Pan. **The sensory disturbances in patients stroke combined with small fiber neuropathy.** *Life Sci J* 2013;10(1):3108-3112]. (ISSN:1097-8135). http://www.lifesciencesite.com. 386

Keywords: sensory disturbances ,stroke ,small fiber neuropathy, skin biopsy ,epidermal nerve fiber density.

Introduction:

Paresthesia is common in stroke patients, presenting mainly as pain, numbness, allodynia. These symptoms are prominent and with long duration resulting in poor clinical outcomes and seriously affecting the quality of life of patients. Skin conduction pain nerve fibers having a diameter less than 7μm A-δ-based fibers and C fibers, called the small nerve fibers, these fibers are referred to as a small fiber neuropathy (Small fiber neuropathies, SFN). When lesions occur in these, the symptoms varies to pain, and commonly numbness [1]. The pathogenesis of troke with symptoms of paresthesia, small fiber neuropathy is unclear. The skin biopsies technology is the gold standard for the diagnosis of small fiber neuropathy. Joanna Wallengren [2] reported case of post-stroke hemiplegic side distal upper limb in patients with pruritus, found its itching side epidermal nerve fiber density (intraepidermal nerve fiber density, IENFD) decreased. To further explore the sensory dysfunction after stroke whether there is damage to nerve fibers, this study collected paresthesia stroke patients and a control group of 20 cases. The surrounding skin, were observed within the epidermis nerve fiber morphology, counted IENFD in the number of samples, and t test and SPSS statistical software between the two groups and the group the differences between different gender were applied

Materials and Methods

1.1 Study was conducted from January 2012 to December 2012, in the First Affiliated Hospital of Zhengzhou University, Department of Neurology, with presence of sensory disturbances stroke patients. Diagnostic criteria [3]: a variety of causes which resulted in blood supply of the brain disorder, acute and chronic onset of focal or whole brain dysfunction, lasting more than 24 hours, or imaging brain lesions associated with clinical symptoms. Inclusion criteria: (1) meet the diagnostic criteria (2) the presence of numbness, pain and temperature sensation sensory disturbances or autonomic nerve damage performance, with or without weakness. Exclusion criteria [3]: (1) serious liver and kidney dysfunction and blood system diseases (2) transient ischemic attack (TIA), traumatic brain injury, subarachnoid hemorrhage patients (3) clearly can affect the pre-hospital check and who could not cope with peripheral nerve damage diseases: diabetes, AIDS, skin Sjogren's syndrome (4) hereditary neuropathy (5) refused to participate in the research.

Stroke Group: 12 males, 8 females, mean age 51.20 ± 12.58 years old. [4]: race, age, gender, height, weight, detailed neurological physical examination to collect general feeling focused inspection (including vibratory sensation, position sense, pinprick, kinesthesia, touch, pain and temperature sensation), liver and kidney function, electrolytes, blood lipids, blood glucose (if necessary, line OGTT), glycosylated hemoglobin, vitamin B12, and folic acid. Normal control group: 12 males, 8 females, average age 42.85 ± 14.30, after the statistical analysis, the two groups in age with no significant difference.

1.2 Research Methods: Local disinfection of alcohol, 2% lidocaine anesthesia, take the ring with a diameter of 3mm the hole vertical burrow into the skin (avoid local anesthetic injection the pinhole parts), a depth of about 2-3mm, the tweezers gently gripping the skin, scalpel blades separation below the organization, take the lower extremity distal skin

(lateral malleolus 10cm) [1], put 12-24 hours to 2% PLP fixative fixed, frozen sections perpendicular cut thickness 40μm slices, put to the 96-well plate, PGP9.5, immunohistochemical staining, marked peripheral nervous system (peripheral nerve system, PNS) neurons form of count IENFD. IENFD refers to the number of units of of epidermal length or units epidermal area PGP9.5 positive nerve fibers. Under the microscope a different magnification (× 40 × 100 × 400) camera different focusing state were shooting under the micro-scale images, in order to deal with the photographs taken under different magnifications and different focusing state respective staff.

1-8 each count of a nerve fiber. Through the basement membrane of a nerve fiber branch count 1; 2,3 nerve fibers branch within the basement membrane is counted as one; the 4,5 branch of the nerve fibers in through the basement membrane of each counted as one; 6 nerve fiber through the basement membrane interruption is counted as one; 7 no nerve fiber white plexus issued, but seen through the basement membrane is counted as one; 8 nerve fibers through the basement membrane is counted as one. I and II nerve fibers do not count. I nerve fibers through the basement membrane does not count; II epidermal visible nerve fibers branch but not through the basement membrane does not count.

1.3 Statistical methods for continuous variables and normal distribution test (Kolmogorov-Smirnov test). Between the two groups of normal distribution data are used to compare two independent samples t-test, Levene's test of homogeneity of variance test. Mann-Whitney U rank sum test for non-normally distributed data. Measurement data were expressed as mean ± standard deviation, the difference was statistically significant at $P < 0.05$.

2. Skin biopsy results

2.1 Nerve fiber morphology

Part axonal swelling seen and varicose light microscope, segmental, beaded change, some small branches to reduce partial interruption of nerve fibers. Nerve fibers than leather, issued through the basal layer into the epidermis, the epidermis dermis both branch, unbranched part of the small fibers are present in the epidermis. Figure 4-6.

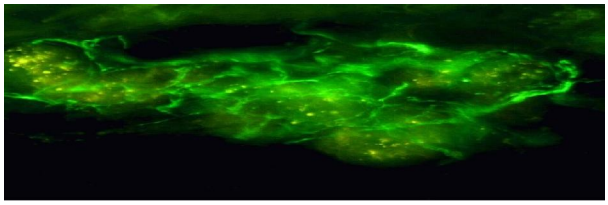

Figure 1. Control sweat glands innervated **PGP9.5 staining, 200 ×**

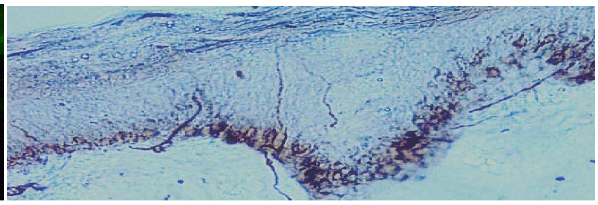

Figure 2 control PGP9.5 staining, 200 ×

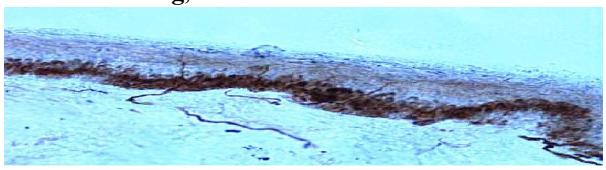

Figure 3 normal control PGP9.5 staining, 200X

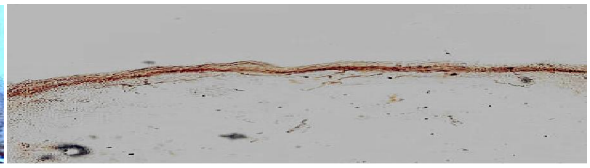

Figure 4 PGP9.5 staining of stroke patients, thinner epidermis 100 × epidermal thickness IENFD reduce interruptions

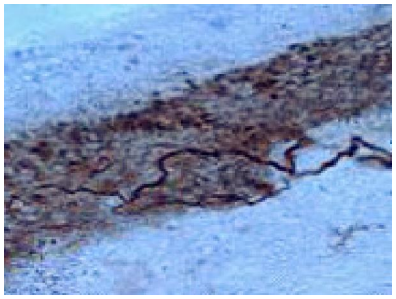

Figure 5 .Stroke patients PGP9.5 staining Nerve fibers swelling, Distorted 200X

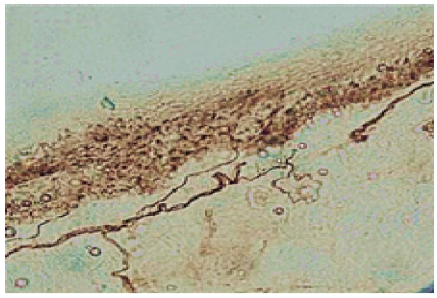

Figure 6. Stroke patients PGP9.5 staining nerve fibers axonal swelling, small branches decreased 400X

Epidermal nerve fiber density

Stroke group compared with the normal control group, a significant decrease in the density of nerve fibers in the epidermis (as illustrated in table 1)

Table 1. Stroke group and normal control group calf epidermal nerve fiber density

Groups	IENFD（Root/mm）	tvalue	Pvalue
Normal control group	14.99±1.84	6.33	<0.01
Stroke Group	11.75±1.37		

Stroke group compared with the control group (P<0.01)

Normal control group comparison between different gender, epidermal nerve fiber density was no significant difference (Illustrated in table2)

Table 2. Normal control group different gender IENFD comparison

Sex	IENFD （root/mm）	t value	Pvalue
Male	15.21±1.90	-0.18	0.86
Female	14.67±1.82		

Comparison between the different normal control group gender wise P>0.05

Stroke group comparisons between different gender, epidermal nerve fiber density was no significant difference (Illustrated in Table3)

Table 3. The comparison of IENFD stroke group gender wise

Sex	IENFD（root/mm）	tvalue	p value
Male	11.70±1.30	0.64	0.53
Female	11.82±1.56		

Comparison of different genders in Stroke Group.

3. Discussion

High incidence of stroke, high morbidity and high mortality, a variety of clinical manifestations, the majority of patients with limb weakness, hemianopia, aphasia, behavioral and psychological abnormalities and disturbance of consciousness, such as treatment admission, pain, numbness, and autonomic nervous system dysfunction often ignored, but its symptoms are of longer duration. With the improvement of life quality requirements, the symptoms more and more to get people's attention. Combined with sensory and autonomic nervous system dysfunction in stroke patients, the majority of the performance of laterality numbness, abnormal urine, sweating or no sweat, with or without limb weakness. Little improvement was seen even given the appropriate treatment. The study included 20 patients, ranging in duration from 4 days to 20 years. Persistent numbness, no sweating, seriously affecting the quality of life was found.

Conduction pain nerve fibers in the skin is mainly a diameter of less than 7μm was A-δ-based fibers and C fibers, known as the small nerve fibers, these fibers occurs lesions called an SFN. According to its diameter, the peripheral nerve fibers can be divided into the ABC three types. Wherein A is divided into four subtypes, A-α, A-β, A-γ, A-δ A-alpha, A-beta, A-gamma belonging to the large diameter fibers. Type B for medium diameter. C-type nerve fibers finest diameter of only 0.5 to 1μm are demyelinated fiber main pass postganglionic fibers of the autonomic nervous system and the body, the afferent fibers of the autonomic nervous system nerve impulses. Medium and large diameter nerve fiber conduction deep feeling and movement can be found by conventional EMG, the small diameter of the myelin sheath A-δ fibers and demyelinated C nerve fiber conduction temperature sensation, pain and autonomic functions, and in most cases EMG cannot be conventionally discovered. Inspection method for the diagnosis of small fiber neuropathy are many, including: skin biopsy, and quantitative sensory testing (quantitative sensory testing, QST), quantify reminder Khan axon reflex test (quantitative sudomotoraxon reflex testing distribution QSART), sympathetic skin response (sympathetic skin responses, SSR), pain evoked potential (contact heat evoked potential, CHEP) [1]. Skin biopsy repeatability, simple to operate, easy to obtain, inexpensive and quantitative indicators of observation, sensitivity, specificity, specificity of 95-97% [12, 13], a sensitivity of 45% to 80% [13, 14], at home and abroad to take advantage of this technology has established a

laboratory standard value. Is considered to be the gold standard for the diagnosis of small fiber neuropathy.

Clear cause of small fiber neuropathy [5-10]: drugs and poisoning, metabolic diseases, immune-related diseases, viral infections, and genetic diseases. The pathogenesis is unclear, might be the the polyhydroxy way nerve hypoxia and ischemia, excessive activation, oxidative stress, nerve growth factor and other nutritional factor deficiency [11]. Vascular diseases such as stroke whether there is a causal relationship with SFN has been decided yet.

The results of this study, see the light microscope axonal swelling and varicose segmental beaded change, no significant decrease in small branches, partial interruption of nerve fibers. Nerve fibers than leather, issued through the basal layer into the epidermis, the epidermis dermis both branch, part of the small fibers are present in the epidermis, unbranched. Compared with the normal controls, IENFD significantly lower (P <0.01), with a significant difference, and Joanna Wallengren [2] similar case reports results. Prior to this, there have been a stroke of the middle cerebral artery territory, internal capsule and parietal epidermal nerve fiber density change [15-17]. Patients with stroke risk factors, including hypertension, diabetes, hyperlipidemia, high hyperhomocysteinemia, smoking, alcohol consumption, these risk factors can be caused by of epidermal vascular morphology and density change [18-23]. Before the stroke occurred even after considering the stroke, peripheral vascular that there is a corresponding change, leading to hypoxic-ischemic changes in epidermal nerve fibers of peripheral nerves. The results of this study show the small nerve fiber disease the combined sensory disturbances stroke patients, which is consistent with the results of many articles cases reported here to increase the sample size, provides the existence of small nerve fiber disease for the combined sensory disturbances stroke patients Preliminary statistical data, and provide a basis to further explore the mechanism.

Statistical analysis between the studies suggest that stroke group and different gender in the healthy control group (P> 0.05), the difference was not statistically significant. Some studies have shown [24] about the differences between the sexes, there was significant difference between normal populations of different gender IENFD men grow older, compared with women, IENFD decline. The rest of the studies show no statistical difference between different gender IENFD. This study do not agree with the heavier patients or older patients enrolled, led to the inclusion of cases there is a bias, no comparison between age group, the future may further increase the sample size, the statistical analysis.

Corresponding author:
1. Prof. Dr. Yuming Xu, M.D.,
Director, Department of Neurology,
First Affiliated Hospital of Zhengzhou University, East Jianshe Road,
Zhengzhou, Henan 450000, China
Email- xuyuming@zzu.edu.cn

2. Baohan Pan,
Johns Hopkins School of Medicine,
Baltimore, MD. USA.

References

1. Devigili G, Tugnoli V, Penza P, et al. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology.Brain, 2008, 131 (Pt7): 1912-1925
2 Joanna Wallengren,, Frank Sundler.Cutaneous sensory nerve fibers are decreased in number after peripheral and central nerve damage.J Am Acad Dermatol .. 2002, Feb; 46 (2) :215-7.
3 Chinese Neuroscience Association ,China Association of Neurological Surgeons. Various types of cerebrovascular disease diagnostic points [J]. Journal of Neurology, 1996, 29 (6):379-380.
4. Maria Nebuchennykh, Sissel Løseth ÆSigurd Lindal, Svein Ivar Mellgren.The value of skin biopsy with recording of intraepidermal nerve fiber density and quantitative sensory testing in the assessment of small fiber involvement in patients with different causes of polyneuropathy. J Neurol (2009) 256:1067-1075
5. Boger MS, Hulgan T, Haas DW, et al. Measures of small-fiber neuropathy in HIV infection. Auton Neurosci 2012; 169:56-61.
6. Giannoccaro MP, Donadio V, Gomis Perez C, et al. Somatic and autonomic small fiber neuropathy induced by bortezomib therapy: an immunofluorescence study. Neurol Sci 2011; 32:361-363.
7. Tan IL, Polydefkis MJ, Ebenezer GJ, et al. Peripheral nerve toxic effects of nitrofurantoin. Arch Neurol 2012; 69:265-268.
8. Ferrari LF, Levine JD. Alcohol consumption enhances antiretroviral painful peripheral neuropathy by mitochondrial mechanisms. Eur J Neurosci 2012; 32:811-818.
9. Plante-Bordeneuve V, Said G. Familial amyloid polyneuropathy. Lancet Neurol 2011; 10:1086-1097.
10. Hoeijmakers JG, Han C, Merkies IS, et al. Small nerve fibres, small hands and small feet: a new syndrome of pain, dysautonomia and acromesomelia in a kindred with a novel NaV1.7 mutation. Brain 2012; 135:345 -358.

11. Perkins BA, Greene DA, Bril V. Glycemic control is related to the morphological severity of diabetic sensorimotor polyneuropathy. Diabetes Care. 2001 Apr; 24 (4) :748-52
12. Smith AG, Howard JR, Kroll R, etal. The reliability of skin biopsy with measur-ment of intraepidermal nerve fiber density. J Neurol Sci, 2005,228 (1): 65-69.
13. Justin C, McAthur, MBBS, etal.Epidermal nerve fiber density: normative reference range and diagnostic efficiency [J]. Arch Neurology, 1998,55 (2) :1513-1520.
14. Chien HF, Tsenq TJ, Lin WM, etal. Quantitative pathology of cutaneous nerve terminal degeneration in the human skin. Acta Neuropathol, 2001,102 (5): 455-461.
15. King CA, Huff J, Jorizzo JL. Unilateral neurogenic pruritus: paroxysmal itching associated with central nervous system lesions. Ann Intern Med 1982; 9:222-3.
16. Massey EW. Unilateral neurogenic pruritus following stroke. Stroke 1984; 15:901-3.
17. Shapiro PE, Braun CW. Unilateral pruritus after a stroke. Arch Dermatol 1987; 123:1527-30.
18. Virdis A, Taddei S.How to evaluate microvascular organ damage in hypertension: assessment of endothelial function. High Blood Press Cardiovasc Prev. 2011 Dec 1; 18 (4) :163-7.
19. Vas PR, Green AQ, Rayman G. Small fibre dysfunction, microvascular complications and glycaemic control in type 1 diabetes: a case-control study.Diabetologia. 2012 Mar; 55 (3):795-800.
20. Petrofsky JS. The effect of type-2-diabetes-related vascular endothelial dysfunction on skin physiology and activities of daily living. J Diabetes Sci Technol. 2011 May 1; 5 (3) :657-67.
21. Doupis J, Rahangdale S, Gnardellis C, Pena SE, Malhotra A, Veves A. Effects of diabetes and obesity on vascular reactivity, inflammatory cytokines, and growth factors. Obesity (Silver Spring). 2011 Apr; 19 (4): 729-35.
22. Francischetti EA, Tibirica E, da Silva EG, Rodrigues E, Celoria BM, de Abreu VG.Skin capillary density and microvascular reactivity in obese subjects with and without metabolic syndrome. Microvasc Res. 2011 May; 81 (3): 325 - 30.
23. Sørensen LT, Jørgensen S, Petersen LJ, Hemmingsen U, Bülow J, Loft S, Gottrup F. Acute effects of nicotine and smoking on blood flow, tissue oxygen, and aerobe metabolism of the skin and subcutis.J Surg Res. 2009 Apr; 152 (2) :224-30.
24. Goransson LG, Mellgren SI, Lindal S, et al. The effect of age and gender on epidermal nerve fiber density [J]. Neurology, 2004, 62:774 ~ 777.

3/3/2013