

Article

Biochemical and Surface Ultra-Structure Investigation of Cholesterol in Local Points and Yamamoto New Scalp Acupuncture (YNSA) for Low Back Pain

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Abstract. Low back pain (LBP) is one of the diseases suffered by people over the world due to musculoskeletal disorders. Blood serum was analyzed for total cholesterol, LDL-C, HDL-C, and triglyceride levels. Cholesterol surface ultrastructure analysis was performed using a SEM microscope. The results showed that total cholesterol and LDL-C levels decreased in the blood serum of LBP patients who had undergone acupuncture therapy by 10 times. Morphology of the surface ultrastructure of cholesterol in the blood serum of LBP patients also showed a reduction in the amount of cholesterol after acupuncture therapy by 10 times. These two results might be useful to improve scientific information for reducing LBP pain, especially through acupuncture therapy.

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1. Introduction

LBP is one of the musculoskeletal disorders caused by poor body activity, which is localized under the angle of the last ribs and upper fold of the lower buttocks with or without pain in the legs [1]. Due to its growing prevalence in the world, it becomes a common systems in every nation in the world [2]. This condition showed might be it will be a big amount of money spent to treat this back pain disease [3].

LBP can be caused by many factors including pathophysiology of human itself. Then, this systems can be called by unique disease because of its difficult characteristic to detect at first

examination. Later, LBP can be caused by physical disorders such as trauma, or the other ones such as inflammation, tumors, degeneration diseases, organ abnormality, and physiological factors [1].

Acute muscular LBP is one kind of trauma-caused of LBP. This is caused by external forces so that make the muscle to be injured. When this phenomenon occurs many times, it can be called by chronic LBP. Later, type of LBP that caused by inflammation can be caused by physical injury at vertebrae bodies, such as bamboo. A kind of tumors that have malignant characteristic can be causing LBP because of its metastasis capability. Next, increasing age of man can be caused LBP correlated with degeneration condition of its injured organs connected with LBP. Last, LBP can be caused by another factor such as abnormality in the gallbladder and stomach or reproductive organs [1].

Treatments for LBP are vary, correlated with its kind of LBP occurred to human. If the cause of LBP is tumor accordingly the its best treatment is surgery. Dissection at source of LBP is the main point for curing LBP for tumor causing so that its malignant characteristic will not further occurs. Another choice for treatment tumor-caused LBP is advice for taking antitumor drugs or chemotherapy. Then, if the LBP complain is still low, the conservative treatment is the best to taken, such as application of corset or body warmer [4].

Several studies related to LBP showed the effectiveness methods for decreasing and even treating this disease, such as application manual and electro acupuncture to relief the pain of this disease although there was no significant differences between two those methods [5]. A single acupuncture through Jingjin model (meridian sinew) could be used to relief the LBP disease approximately since five months after treatments [6]. The curative treatment through combination between acupuncture and spinal manipulative therapy was reported not to be the best methods for curing this pain compared to those therapy alone [7-9]. The effectiveness of combination local acupuncture and YNSA for LBP treatment was reported. These many reports indicated the possibility of acupuncture to be alternative method for curing LBP disease although not to be the best one statistically.

Cholesterol is known as one content of lipid fraction of human blood that has many useful function in normal condition. This one kind of lipids is often used to identify the state of human health because of its stability compared to triglyceride. For knowing the health condition, this kind of lipid could be extracted from both plasma or serum of blood. But, blood serum provided more reliable results for cholesterol extraction compared to plasma because of its less of water content [7].

The presence of cholesterol in blood serum can be identified in many ways, one of which uses scanning electron microscope (SEM). This technique has been used in detecting the presence of atherosclerotic lesions in mice and cholesterol crystal in cardiovascular disease [10-12]. Until now, there has been yet a study about detecting cholesterol for LBP disease so that it needs to be done. This study aimed to determine the relationship of acupuncture therapy in LBP sufferers, especially focused on surface ultrastructure of cholesterol and biochemical analysis is also carried out to strengthen the results of this study.

2. Experimental Section

Acupuncture Therapy

In giving acupuncture therapy, the research subjects will first take a history related to complaints of LBP. The results of the history will be recorded, then determines the acupuncture points used for therapy. Later, tools and materials is prepared for therapy including filiform needles measuring 0.20 x 18 mm and 0.25 x 25 mm, alcohol swab and handscoen. In the treatment for the first group, the researcher put a handscoen and disinfected the area of the point to be pierced then the research subject is stabbed using acupuncture points according to the group determining the point. Acupuncture needles that have been plugged will be left for 20 minutes and every 5 minutes, the needle will be given manual manipulation by flicking. After the therapy was completed, the acupuncture needles were removed and the area of the puncture site was disinfected again using an

alcohol swab. The acupuncture therapy points that used were Local Points [Acupuncture points used BL 22 (Sanjiaoshu), BL 23 (Shenshu), BL 24 (Qihaihu), BL 25 (Dachangshu), and BL 26 (Guanyuanshu)], and YNSA points [YNSA points that can be used on Somatotopes D, and extra lumbar points H, I. The puncture is performed on one side (Ipsilaterally)].

Blood Lipid Analysis

Blood Lipid Analysis begins with taking blood, separating its components to obtain serum, and analyzing cholesterol [13-14].

Blood collection and serum preparation

Venous blood was collected in a vovutainer without EDTA for several minutes. After that, it was centrifuged at 3000 rpm for 5 minutes. Serum that is in the top layer is taken and used for further analysis

Cholesterol analysis

The serum obtained was put into a sample tube to measure cholesterol levels using a Hitachi 902 tool.

Ultrastructural analysis of cholesterol surface using SEM mikroskop microscope

Analysis of the surface structure of cholesterol using SEM was carried out on blood serum before and after acupuncture. This research begins with vacuuming and drying with a vacuum dryer for a few moments. The specimen is then attached to a stub (holder) inside the Ion Sputtering Device, to coat the surface of the specimen with platinum and the specimen is observed using an SEM microscope (JSM-6510LA).

3. Results and Discussion

It appeared that acupuncture therapy has been shown to reduce levels of total cholesterol and total Low Density Lipoprotein (LDL-C; or just called by LDL) in blood serum (Table 1). In this study, acupuncture therapy carried out by 10 times can reduce cholesterol levels up to 40 unit values, from the number of 180's mg/dl to the 140's mg/dl, while the LDL-C level in blood serum can go down by around 30 unit value, so that it was all under normal conditions.

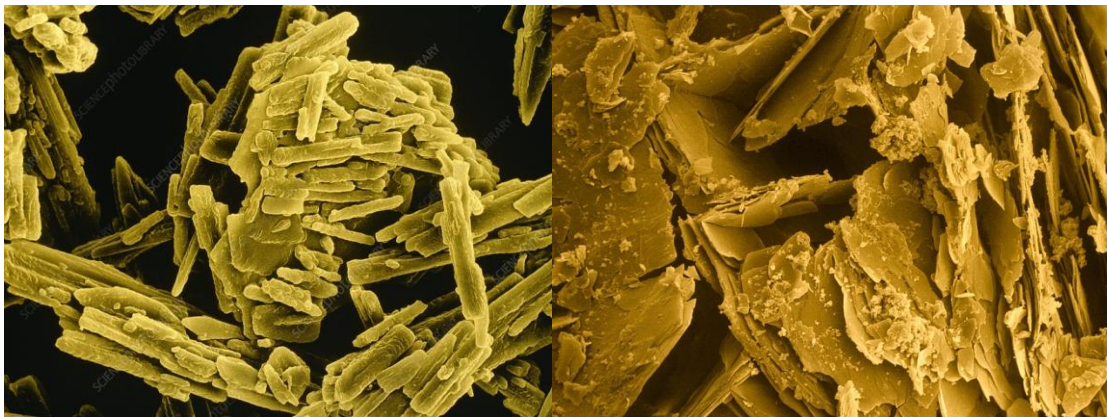


Figure 1. Scanning electron microscopy of cholesterol crystal
(<https://www.sciencephoto.com/>)

LDL is a type of cholesterol that is not good for health. Biochemically, LDL is a compound derived from Very Low Density Lipoprotein (VLDL); ie a kind of cholesterol that has been reported

to increase the frequency of arterial plaque formation [15]. This can be explained that LDL initiates the formation of “soap scum in pipe” so that the body responds to it as a threat so that a lot of proteins related to inflammation will be produced. Furthermore, the inflammation that forms will result in the formation of plaque, which plaque that forms over time will block the flow of blood flowing through the arteries and cause atherosclerosis [16-18]. This in turn will have an effect by increasing the work of the heart in compensating for these conditions, which in turn will increase blood pressure [19-20].

It is reported that there is a relationship between cholesterol crystals and atherosclerosis, namely cholesterol crystals are the forerunner to the occurrence of atherosclerotic plaques [3][21-22]. Furthermore, this plaque will lead to rupture and the incidence of acute myocardial infarction and stroke [1]. Patients with adverse cardiovascular events are reported to have cholesterol plaques compared to patients with other lesions.

The mechanism of plaque rupture is caused by inflammation and physical changes. This is related to the existence of a natural cell mechanism in the interaction of foreign bodies; in this case bad cholesterol (LDL); which will be eliminated by converting it from cholesterol ester form to free cholesterol by macrophages and smooth muscle cells with the help of certain enzymes. In the response mechanism to foreign bodies, this free form of cholesterol will be given to HDL, but along the way, this process can experience obstacles in the form of disturbances in transportation or others, causing accumulation of free extracellular and intracellular cholesterol which will eventually lead to cell death resulting in the formation of cholesterol crystals [23-25]. The formation of cholesterol crystals is related to the expression of the gene that encodes the interleukin 1- β protein which ultimately produces C reactive protein [11]. Cholesterol crystals that form plaques over time will rupture so that they are carried away by the bloodstream and can block blood flow to the brain, resulting in a stroke [8].

In the context of LBP, there is a possibility that there is a relationship between LBP and the amount of abnormal blood lipids that eventually lead to atherosclerosis, especially in the section between the arteries and the aorta, thereby affecting the columnar section. It is further explained that this association may be due to a lack of nutritional supply in the lumbar region resulting in lesions. Furthermore, An association between arterial damage and degenerative disc column disease so that reported that surgery was needed to repair aortic obstruction.

This study did not differentiate the gender of the respondents which was subsequently associated with LBP. But reported that there is a difference between them, namely women who have abnormal blood lipids are more susceptible to attacks of LBP. This cannot be explained naturally but there may be a correlation between the intensity of pain and the longer duration of time in female LBP patients than in male patients.

Table 1. Total cholesterol measurement

No	Examination	T-0	T-5	T-10	Information
1	Total cholesterol	184,4	171,2	149	Cholesterol levels go down
2	Triglyceride	172,2	131,4	166	Triglyceride levels tend go to up and down
3	HDL-C	50	50,8	50	HDL-C levels are stable
4	LDL-C	103,2	94,2	75,4	LDL-C levels go down

Acupuncture is known as an alternative treatment in healing various diseases with the main focus of stimulating meridian points in humans. Some research results showed that acupuncture therapy has a positive correlation in reducing or even curing the severity of various diseases, such as rheumatoid arthritis, coronary heart disease, and migraine. In the field of LBP, acupuncture therapy

has been reported to have a positive impact in reducing the severity with certain methods such as stabbing at the points of "Lumbar Pain Points" and "Traditional Chinese Medical" (TCM).

Characteristics of "healthy cholesterol", HDL-C or just called by HDL, appeared to be stable, which was in the range of 50 mg/dL, so that it can be concluded in the normal range of healthy human level. However, triglyceride levels of blood serum tended to fluctuate but it was still in the normal range, but tend to be high warning.

Normal total cholesterol levels in human blood serum range is less than 200 mg/dL for the normal category, normal LDL-C levels that is less than 100 mg/dL, normal HDL-C levels range is from 40-59 mg/dL, and above it showed that HDL-C is good for the body, and normal triglyceride level is less than 150 mg/dL. Acupuncture therapy with a combination of local points and YNSA by 10 times has been proven to reduce total cholesterol and LDL-C levels so that it is in the normal range. This was likely due to the activation of several enzymes related to oxidative stress due to the stabbing. The oxidation nitrite enzyme as one of the enzymes related to oxidative stress is involved in reducing cholesterol levels [26-29].

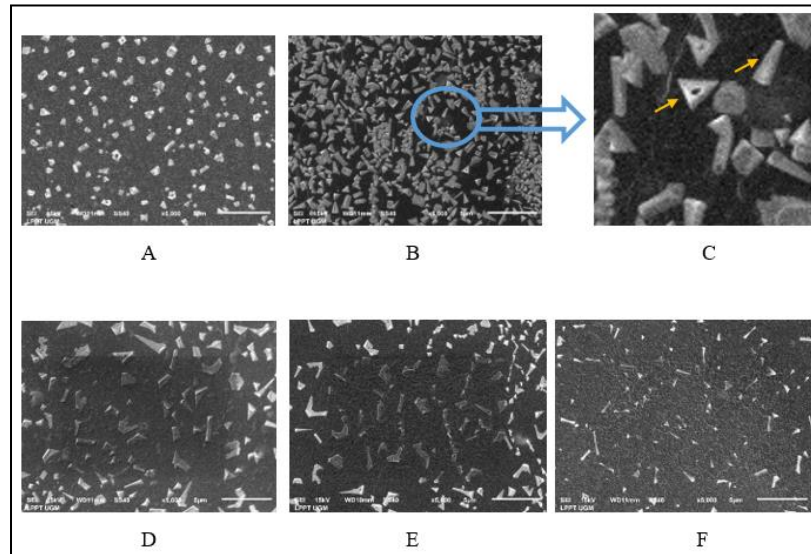


Figure 2. Surface ueltrastructure of cholesterol surfaces in the blood serum of LBP patients. Healthy control (A); pain control (B); magnification indicating cholesterol (C); cholesterol in blood serum in 0 (D) therapy; 5th (E); and 10th (F).

It appears that the amount of cholesterol in healthy controls is relatively small compared to control pain (Figure 2). This can be seen by the structure of cholesterol with a unique morphology such as broken glass. The cholesterol crystals in arterial plaque looked like broken glass. The results showed that cholesterol has a needle-like shape and broken glass with a tapered part at the end and there was also a triangular shape morphology with a hole in the middle. The cholesterol that isolated from human monocyte derived macrophages and human aortic endothelial cells was likely to plate, needle, and hair-pin sharp forms [30]. Some of these cholesterol morphologies appeared to decrease in blood serum in patients who have undergone 10 times acupuncture therapy [31]. Thus, morphologically surface ultrastructure can be proven that acupuncture therapy can reduce blood cholesterol levels in LBP sufferers.

4. Conclusion

Acupuncture therapy ten times has been proven to reduce total cholesterol and LDL levels in LBP sufferers. In addition, HDL and triglyceride levels in LBP sufferers also tended to be in the normal range after 10 times acupuncture therapy. Surface ultrastructure analysis of cholesterol surfaces in the blood serum of LBP patients showed a reduction in the amount of cholesterol.

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