The Acupuncture Effect for Low Back Pain; Biochemical and Protein Profile Analysis

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Article Info

Abstract

Low back pain (LBP) is one of the common symptoms experienced by some of the world community. Acupuncture as a method of healing a disease offers a solution for LBP symptom. This study aimed to determine the effect of acupuncture with local and Yamamoto New Scalp Acupuncture (YNSA) points with biochemical analysis related to total protein, albumin, and globulin, and analysis of blood serum protein profiles using the SDS-PAGE method. This research was conducted using sterile acupuncture needles that inserted in patients with several local points, namely BL22 (Sanjiaoshu), BL23 (Shenshu), BL24 (Qihaishu), BL25C (Dachangshu), and BL26C (Guanyuanshu) and combined with the YNSA points through somatotope D points and extra lumbar points H and I. Blood serum was collected for total protein, albumin, and globulin analysis. That blood serum was also used for protein profile analysis using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method. The results showed that six times of acupuncture treatments gave positive correlation in total protein, albumin, and globulin. Analysis of blood serum protein profiles using SDS-PAGE showed a protein band of about 12 KDa and it might be a interleukin-13 protein. The results were expected can be useful as scientific information especially related to acupuncture for LBP treatment and this of about 12 KDa protein band can be used as biomarker candidates for the symptoms.

Introduction

LBP is a common complaint or symptom of musculoskeletal for everyone. This symptom generally occurred from the lower rib margins and the buttock creases (Dionne et al., 2008)and limitations or impossibilities in comparing or summarizing prevalence figures from different studies. Back pain definitions were identified from 51 articles reporting population-based prevalence studies, and dissected into 77 items documenting 7 elements. These items were submitted to a panel of experts for rating and reduction, in 3 rounds (participation: 76% and this is to be one of the most causes of disability in the world (Vos et al., 2016)a trend largely attributable to an epidemiological transition in many countries from causes affecting children, to non-communicable diseases (NCDs. LBP often occurred in the medium to low end income of society such as in Asia and Africa people (Hoy et al., 2015)limitations and lessons learned from estimating the burden from musculoskeletal (MSK. In Indonesia, musculoskeletal disorders were many reported, one of them was in batik workers (Sumardiyono & Ada, 2014).

Acupuncture therapy uses effective and significant puncture points to reduce LBP. This is caused by the pricking of acupuncture points at the particular points which can affect the nociceptive, proprioceptive and autonomic nerve pathways. Acupuncture therapy can increase encephalin and dinorphine in the local area around the waist and at the same time have a segmental effect to send impulses to the midbrain so that it can increase endorphins in the pituitary hypothalamus. Encephalin flow in the midbrain can also stimulate the release

of monoamine, serotonin and norepinephrine in the lower waist so that it can inhibit pain, including musculoskeletal pain (Audette & Ryan, 2004)arthritis, carpal tunnel syndrome, fibromyalgia, and upper extremity tendinitis. Randomization, appropriate sample size, and blinding using more sophisticated sham procedures raise the quality of the studies from a scientific, methodologic point of view. In addition, realistic treatment frequency and duration of some of the more recent studies have resulted in more favorable outcomes. Much work still has to be done, however, to find ways to preserve the clinical authenticity of acupuncture treatment methods when brought into the light of a research protocol. Attempts have been made to find a method of maintaining the standardization and reproducibility of a research protocol, while allowing the kind of flexible treatment that normally would be applied in a clinical setting. Other questions that should be answered with future studies include understanding how treatment length influences outcome, if maintenance treatments are needed for chronic conditions, and cost and risk comparisons with standard pharmacologic treatment. In addition, future studies need more overt statements of the rationale for the treatment method used (eg, were Chinese or Japanese diagnostic methods used for point selection, what needling technique was used, was the De Qi sensation elicited.

Several local points of acupuncture, such as BL-22, BL-23, BL-24, BL-25, and BL-26, were reported to be one choice for curing LBP (S. H. Lee et al., 2013). Next, related to LBP Japanese practice of craniopuncture microsystem, YNSA, is differ to Chinese one called by Chiao Shun Fa. This YNSA method of puncture only a tip of needle and concentrated in the forehead. Even though it seems simple method but it reported to be more effective in reducing pain compared to Chiao Shun Fa (S.-J. Lee, Shin, Lee, Han, & Kim, 2013).

Acupuncture combination therapy between local points and YNSA has a dual effect in reducing low back pain. Acupuncture therapy using a combination of local points and YNSA works through four domains, namely: 1) local inflammatory reaction, 2) intercellular meridian transduction, 3) cutaneosomatoviscera reflex,

and 4) neural transmission to the brain (neuro acupuncture). Local inflammatory reactions are characterized by vasodilation. Intercellular transduction of meridians is characterized by the exchange of electric ions in the meridian pathway. The cutaneosomatoviscera reflex is characterized by activating the pain modulation system by suppressing the transmission and perception of pain stimulation at different levels in the central nervous system. Therefore, this combination of therapies is excellent in decreasing musculoskeletal pain (Gellman, 2014; Ma & Cho, 2004; Saputra & Sudirman, 2009).

Serum is known as part of blood after removing of clotting factor, on the other hand, blood plasma is still there. Burtis & Ashwood (2001)reported that serum contains macromolecules such as proteins (amino acids), carbohydrates (sugars), and lipids. Albumin and globulin especially immunoglobulins make up blood serum, next to secreted and synthesized proteins (Kennedy, 2001; Schrader & Schulz-Knappe, 2001). Geyer et al., (2017) classified serum protein into three classes, i.e related to house-keeping function such as albumin, related to oxidation condition such as aspartate aminotransferase, and related to hormons such as cytokines. Both serum and plasma were reported to be important for biomarkers of many diseases(Dayon & Kussmann, 2013; Rajandram et al., 2014; White et al., 2014), but the diagnosis using serum is more accurate due to the absence of a blood clotting factor.

The biochemical parameters can explain the mechanism of pain, establish diagnosis and handle it appropriately. One measurement that can determine the therapeutic effect of pain management is to look at the biochemistry of one kind of protein called by cytokines or endorphins. Cytokine measurements can explain the mechanism of pain and subsequent actions in patients (Kaufman & Carl, 2013). Siswantoyo (2012) reported that exercise stressors can increase beta endorphin levels by 4.24 ng.ml^{-1}. To date, biochemical analysis of acupuncture therapy in patients with LBP has not been reported yet.

A human's health condition can be seen in various ways such as using protein analysis. This analysis can show a disturbance that

occurs in someone they are not even aware of because it gives a description of protein activity in a human's body at a certain time. Thus, by comparing with healthy and sick human as positive and negative control respectively, it can be seen that proteins can be as a biomarker candidates for certain diseases. One method of protein analysis that is simple and can provide a general description of human health is the SDS-PAGE method. This method although included into traditional method but is now still widely used (Adhitya, Ernawati, & Plumeriatuti, 2013; Liu et al., 2015).

Measurement of serum protein profiles in LBP cases to date has also not been reported yet. Based on these facts, it is necessary to conduct biochemical analysis and characterization of serum protein profiles in LBP patients receiving acupuncture therapy to investigate of total protein, albumin, and globulin for biochemical analysis and SDS PAGE method for serum protein profiles analysis.

Methods

Acupuncture treatment with local and YNSA points was done with sterile acupuncture needles that are inserted in patients with several local points, namely BL22 (Sanjiaoshu), BL23 (Shenshu), BL24 (Qihaishu), BL25C (Dachangshu), and BL26C (Guanyuanshu). That acupuncture therapy later was combined with the YNSA points through somatotope D points and extra lumbar points H and I.

Retrieval of serum protein samples was carried out through the brachial vein using an intravenous serum that had anticoagulant ethylene diamine tetra acetic acid (EDTA) or heparin at 0 and 6th times of acupuncture treatments. The blood was taken as much as 5 ml and then stored in the box to be stored further in the temperature of $-20°C$ until the next step of protein extraction was started.

Blood serum of patients was investigated for its total protein, albumin, and globulin with Sinnowa 3000P machine. That blood serum was also investigated for its protein profiling using SDS-PAGE methods. Protein separation was done using SDS-PAGE method that consists of three stages, namely 1) protein extraction stage, 2) gel making and 3) protein separation through 1-D electrophoresis. Protein extraction was started by addition of ethanol solution on

samples following the centrifugation step at 13000 rpm. Supernatant was used for further analysis and called as soluble protein. Soluble protein concentrations were determined using the Bio-rad method. For SDS-PAGE analysis, protein samples along with markers each with a final volume of 25 μL and 5 μL were injected into gel well and electrophoresis was performed at 100 volt for 2 hours. The next steps were staining with Coomasie brilliant blue 0.1%, destaining, and storage in glacial acetic acid 10%. Analysis of the protein profiles that appear on the SDS-PAGE gel was carried out by identifying the molecular weight (MW) of protein bands formed in each sample and analyzed using ImageJ software (https://imagej.nih.gov/ij/).

Results and Disscussions

In general, it can be seen that acupuncture therapy as many as six times can normalize total protein, albumin, or globulin. Normal level of total protein is around 6.0 to 8.0 $g.dL^{-1}$, normal level of albumin is around 3.5-5.0 $g.dL^{-1}$, and normal level of globulin is around 2.5-3.0 $g .dL^{-1}$ (Busher, 1990). Normal levels of total protein, albumin, and globulin will have an impact on improving the quality of health, which in this study is related to LBP.

Albumin and globulin in blood serum can be a sign of one's health. Albumin is known as one of the proteins contained in the blood and is produced in the liver. Next, globulin is also produced in the liver and is included in one component of self-defense. Both globulin and albumin are globular proteins (fetoproteins) that have many functions for body, such as catalisator (enzymes), signal transduction, and regulation.

The low ratio of both was reported to be related to the presence of breast cancer (Azab et al., 2013) and stomach cancer (Bozkaya et al., 2018). This condition was based on the fact that albumin and globulin constitute the largest portion of blood serum (K. Li, Fu, Bo, & Zhu, 2018) and albumin can be correlated with inflammatory condition (Ferrer et al., 2018). It was also thought that globulin can be a marker for inflammation due to the fact that some pro-inflammatory related proteins are present in globulin such as interleukin (interleukin 6 and 1β) and tumor necrosis factors (G. Li et

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Tabel 1. Measurement of Total Protein, Albumin, and Globulin before and after Acupuncture Treatments

No	Name	Measurement	Total Protein	Albumin	Globulin	Evidence
1.	Mrs. A	Before	7.4	5.0	2.4	Total protein at below normal level
		After	7.2	4.5	2.7	Normal level
2.	Mrs. B	Before	8.0	4.5	3.5	Total protein at high level
		After	8.2	5.0	3.2	Trend globulin from high level --> normal
3.	Mrs. C	Before	7.2	4.7	2.5	Total protein at below normal level
		After	7.0	4.2	2.8	Normal level
4.	Mr. A	Before	7.2	4.8	2.4	Total protein stable
		After	7.2	4.0	3.2	Trend globulin at high level --> normal
5.	Mr. B	Before	8.6	5.4	3.2	Total protein at below normal level
		After	8.1	4.6	3.5	Tren albumin decreased --> normal
6.	Mr. C	Before	6.9	5.4	1.5	Total protein at below normal level
		After	6.8	4.6	2.2	Tren albumin decreased. Globulin increased --> normal

al., 2018; Simó, Barbosa-Desongles, Lecube, Hernandez, & Selva, 2012). Next, one kind of globulin, IgE, was also reported has positive correlation with allergy on poultry's workers (Wijayanti, Sutomo, Astuti, & Asmara, 2018).

Acupuncture was also to be one choice for LBP's treatment with varieties of its methods. The local point methods treatment was reported to be important to LBP patients in Korea following to distal point on the meridian of pain area(Yong-Suk, 2010). Matsubara et al., (2011) was also reported the benefit of local and distal points method of acupuncture for chronic neck pain in females. In same hand, YNSA method was also reported to be more effective than sham treatment on acute non-specific LBP (Hasegawa, Baptista, de Souza, Yoshizumi, & Natour, 2013). A combination of local points and YNSA was used in this study based on Yatmihatun, Badri, & Wardoyo, (2019) that showed combination of local points and YNSA method can reduce LBP pain faster than the treatment of each of the two types of therapy. These all studies proved both local points and YNSA acupuncture were useful for back pain.
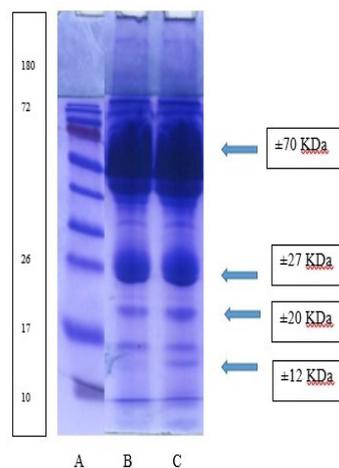

Figure 1 Protein profile using SDS-PAGE of sick and healthy control from human blood serum. Marker (A); sick control (B); healthy control (C).
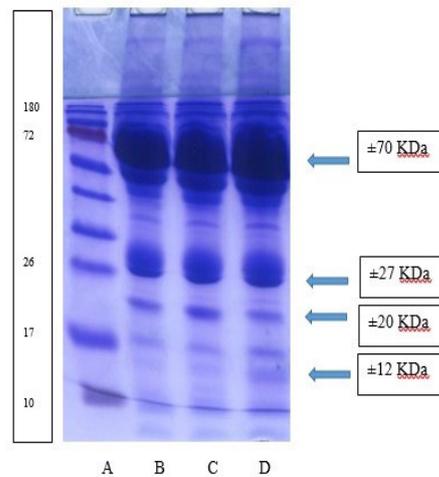
Figure 2 Protein profile using SDS-PAGE before acupuncture therapy, Marker (A); samples (B-D)
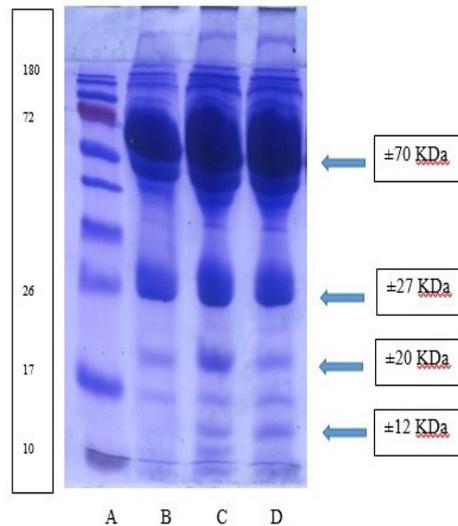

Figure 3 Protein profile using SDS-PAGE after acupuncture therapy, Marker (A); samples (B-D)

Protein bands with molecular weight of around 12 KDa were not seen in sick controls but were clearly seen in healthy control (Figure 1). This result showed the possibility of anti-inflammatory related protein was occurred in healthy control so that there were many anti-inflammatory related proteins that were estimated to be of about 12 KDa. The similar results were also seen in LBP patients' blood serum before and after acupuncture treatment as much as six times (Figures 2 and 3), i.e. enhancement in the intensity of the protein band thickness of about 12 KDa in blood serum that had experienced acupuncture therapy as much as six times. This was predicted along with an enhancement in the amount of protein associated with anti-inflammation protein due to the reduction in inflammation.

A protein with a molecular weight of around 12 KDa is thought to be a candidate of biomarker of healthy control and patients with LBP. This band of around 12 KDa is thought to be an interleukin 13 protein known as anti-inflammatory protein. These proteins were included into cytokines members and were reported to be made by TH2 cells and secreted

by helper T cells, mast cells, NK cells (Gomes et al., 2016). These proteins were closely related to anti-inflammatory protein which has been proven in vitro and ex vitro.

Interleukin was one kind of cytokines that associated with immune system. One kind of interleukin, Interleukin 13, was reported to be associated with innate immunity type-2, next to interleukin 4 (Bao & Reinhardt, 2015) and belongs to anti-inflammatory cytokines. Locati et al., (2013) was reported that interleukin 10 was also associated with them. On other hand, interleukin [IL]-1β, IL-6, and tumor necrosis factor alpha (TNF-α) were reported to be pro-inflammatory interleukin on systemic lupus erythematous (Umare et al., 2014).

Interleukin 13 was reported to be associated with a reduction in the risk of cardiac injury due to viral myocarditis through activation of M2 macrophage cells (Yang, Chen, & Gao, 2017). The amount of this protein was correlated to asthma and diseases related to eosinophil cells (Doran et al., 2017). Martinez-Nunez, Louafi, & Sanchez-Elsner, (2011)pro-Th 1 also reported that interleukin-13 was also involved in human macrophages immunity.

Furthermore, for constitutive proteins, there were at least 3 protein bands, which were of around 20 KDa, 27 KDa, and 70 KDa. The three protein bands were thought to be stress-related types of proteins (heat shock proteins) i.e. Hsp20, Hsp27, and Hsp70. The Hsp20 and Hsp27 proteins are also known as HspB6 and HspB1 which are one type of small Hsp protein classified as Class I. The main difference between the two was Hsp20, that was found in muscles so that it was closely related to muscle relaxation, whereas Hsp27 is found in the cytoskeleton (Bakthisaran, Tangirala, & Rao, 2015)keeping them in a folding-competent state and refolding them by themselves or in concert with other ATP-dependent chaperones. Mutations in human sHsps result in myopathies, neuropathies and cataract. Their expression is modulated in diseases such as Alzheimer's, Parkinson's and cancer. Their ability to bind Cu^{2+}, and suppress generation of reactive oxygen species (ROS. Hsp20 was reported to play a role in the protection of hepatocellular carcinoma (HCC) by blocking the proliferation stage through the formation of a cascade cascade on HCC cells (Nagaswara et al., 2014). Hsp 20 and Hsp27 proteins were reported to have a function in apoptosis, namely in the event of Hella cell apoptosis inhibition through inhibition of cytochrome c release from mitochondria and activation of caspase 3 (Nahomi, DiMauro, Wang, & Nagaraj, 2015) when the cells were thermally stressed, the peptide was translocated from the cytoplasm to the nucleus. The two peptides inhibited apoptosis in HeLa cells by blocking cytochrome c release from the mitochondria and caspase-3 activation. We found that scrambling the last four amino acids in the two peptides (KAIV in Hsp20 and KTLV in Hsp27. In addition to the two proteins, the Hsp70 protein was also thought to be a constitutive protein in this study. This protein was found in the cytosol, nucleus, endoplasm and mitochondrial reticulum, and also extracellular (Turturici, Sconzo, & Geraci, 2011). This protein was also reported to have a role immunomodulation function and related to histocompatibility complex (MHC) in human (Radons, 2016)protein import into organelles, recovering of proteins from aggregation, and assembly of multi-protein complexes. These chaperones augment organismal survival and longevity in the face of proteotoxic stress by enhancing cell viability and facilitating protein damage repair. Extracellular HSP70s have a number of cytoprotective and immunomodulatory functions, the latter either in the context of facilitating the cross-presentation of immunogenic peptides via major histocompatibility complex (MHC.

Electrophoresis is known as a protein separation method and can be used for early detection of a disease. Protein separation using the electrophoresis "Sebia" method can be used in the pathological detection of human blood serum (Rostini & Rita, 2009). Nevertheless, SDS-PAGE as one of the simple electrophoresis methods, can also be used for getting scientific information about disease or symptom, and here we proved that the SDS-PAGE can be useful for that especially in LBP symptom.

Conclusions

Six times acupuncture therapy using combination of local and YNSA points can normalize total protein, albumin, and globulin in LBP patients. Protein analysis using SDS-

PAGE showed that there was of about 12 KDa protein bands which later might be used as a biomarker candidate in LBP.

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