PHYSIOLOGICAL EFFECTS OF SPINAL MANIPULATION IN UNIVERSITY STUDENTS FROM 20 TO 30 YEARS OLD PRE AND POST TREATMENT

Vertebral manipulation and its effect on immunoglobulins

De Nova-Carmona CE¹, Cortes-Altamirano JL^{1,2}, Herrera-Lopez ME¹, Bandala C^{1,3}, Godínez-Sanchez OJ¹, Romero-Morelos P³, Montiel-Flores E^{1*}

¹ Universidad Estatal del Valle de Ecatepec, UNEVE. Ecatepec de Morelos, Estado de México, México. ³Instituto Politécnico Nacional, IPN. Ciudad de México, México.

Abstract - In recent years research focused in showing the effects of spinal adjustments on the immune system has been grown. Changes in immune biomarkers have been reported after spinal manipulation. Immunoglobulins are the body's defense mechanism reflecting the individual's health status and their response ability to disease. The collaborative relationship between the nervous system and the immune system results in an efficient defense mechanism. The main objective of this study was to evaluate the levels of immunoglobulins before and after spinal adjustment in young people. Twenty-six healthy young people with an average age of 20 years were included, all study participants had radiographs taken before the protocol as well as at the end of the protocol. The study subjects were adjusted twice a week with the Thompson technique for 10 weeks. The blood sample was taken before starting the protocol and later a sample was taken after adjustment number 8, 16 and 20. Subsequently, the concentration of IgG, IgM, IgA and IgE monoglybins was evaluated by means of the Elisa test. The results of the study show that there were no significant changes in IgA, IgM and IgE concentration after spinal adjustment, however, a significant change in IgG concentration was observed in women (P=0.02). Spinal manipulation has positive effects on the musculoskeletal system, however, the effect of spinal manipulation on the immune system has not yet been determined. Although results of variability in the concentration of biomarkers of the immune system have been found, these do not exceed physiologically normal levels in young, healthy individuals.

Keywords: Immunoglobulins, Vertebral adjustment, Thompson, Chiropractic, Youth.

I.- INTRODUCTION

Immunity is the body's innate ability to manage pathologies, throughout cellular mechanisms or production of antibodies for self-defense in response to natural exposure or inoculation of infectious agents. (1)

There is a broad collaborative relationship between the nervous and the immune system, generating an efficient defense mechanism. On one hand, the immune system generates a response reaction to infectious agents, this information is received by the nervous system to generate an collaborative response. (2) The nervous system is responsible for focusing the immune response on the specific point of conflict, generating a systemic response. (3) ² Laboratorio de Neurociencias Básicas. Instituto Nacional de Rehabilitación, "LGII". Ciudad de México, México.

*Corresponding Author

The immune system is made up of immunoglobulins also known as antibodies, which are glycoproteins produced by B lymphocytes (receptors of foreign agents) discovered in 1980. Their main function is to recognize, activate a response and neutralize external agents, inactivating their pathogenic activity in the organism through cell membrane receptors, initiating the humoral response. Antibodies are the main line of defense of the humoral immune system. T lymphocytes originate in the bone marrow, however, unlike B lymphocytes, they mature in the thymus and from there are distributed to different tissues. (4,5)

There are 5 types of immunoglobulins (IgA, IgD, IgE, IgG and IgM) which fulfill different functions. These immunoglobulins are always present in the human body but are only activated in the presence of antigens. (6,7)

On the other hand, Chiropractic has been used for several centuries in order to recover health naturally without the intervention of drugs or external agents, only through spinal adjustments. The optimal functioning of the spine depends on the adaptation and physiological interaction of three systems: active (muscular), passive (bone) and control (nervous) (8). The result of this interaction will be reflected if the joint function is effective, if flexibility is sufficient to adopt appropriate postures and alignments under the control of the nervous system, which leads to correct biomechanics, however recent studies have reported that biomechanical alterations in the spine cause neurophysiological dysfunction. (9)

Vertebral adjustments cause changes in different biochemical markers of the organism. Changes in the concentration of substance P, neurotensin, oxytocin and interleukins have been reported, as well as changes in the concentration of cortisol after manipulation. Because these biochemical markers modulate pain and/or inflammation, spinal manipulation is considered a sound strategy for managing conditions that cause pain and/or inflammation. (10)

Thus, chiropractic practices are of great benefit to treat various pathologies in a non-invasive way and without the use of medications, various research has suggested that following chiropractic care could increase the body's ability to produce antibodies, in the same way, it could increase the body's ability to recognize, respond to and remember antigens, as well as increase phagocytic activity. On the other hand, there is scientific evidence that mentions that there are no changes in the activity of the immune system after a chiropractic adjustment (11). For this reason, the main objective of this work was to evaluate the levels of IgG, IgM, IgA and IgE before vertebral adjustment and after vertebral adjustment with the Thompson technique in young people between 20 and 30 years of age.

II.- MATERIALS AND METHODS

An analytical, experimental, longitudinal and prospective study was carried out. A self-controlled group made up of 50 female and male participants between 20 and 30 years of age, without clinical signs of disease, were integrated into the study. All members had 6 radiographs taken (2 cervical spine AP and Lateral view; 2 thoracic spine AP and Lateral view; 2 lumbopelvic spine AP and lateral view) before starting the protocol and at the end of the protocol. The participants were adjusted in the Laboratory of the State University of Valle de Ecatepec, they underwent two vertebral adjustments per week, on Mondays and Thursdays from 8:00 a.m. to 1:00 p.m. for a period of 3 months, with the Thompson technique. A control blood sample was taken before starting the experimental phase, then a blood sample was taken after vertebral adjustment number 8, 16 and 20.

Sampling:

A blood sample was obtained from each patient prior to the chiropractic treatment (time 0), subsequently a sample was obtained in adjustment 8 (first month), in adjustment 16 (second month) and in adjustment 24 (third month). The absorbance (nm) of each sample was obtained, to determine the concentration of Immunoglobulins (μ g or ng) at the different times, the corresponding conversion was applied according to the manufacturer's specifications, finally they were compared with the standards of each kit.

Immunoglobulin analysis procedure.

A blood sample was taken by venipuncture, each sample was obtained with yellow cap vacutainer tubes, then it was centrifuged at 3000 RPM for 30 minutes at room temperature, once the blood components were separated, the plasma (2 ml) was taken and stored in 5 mL tubes at 4°C and the samples were analyzed at 2-3 hours after obtaining it.

All samples were analyzed by spectrophotometric methods, a microplate was used to obtain the Absorbance, Immunoglobulin kits (Abcam, Invitrogen and Mexilab) were used for IgA, IgG, IgD, IgE and IgM and all samples were processed by the method of ELISA. The procedures were carried out under the ethical standards for the research with human beings according to the Declaration of Helsinki of 1975. The participants delivered the Informed Consent Letter for Adults and the Confidentiality Letter based on Article 17 of the Regulations of the General Health Law on Health Research.

Statistical Analysis: Means, deviations and standard errors were determined for the quantitative variables, frequencies and percentages for the gualitative variables. The Kolmogorov-Smirnov test was applied to evaluate the distribution of the quantitative variables. Student's t test was applied to compare two independent means. The repeated measures general linear model test was carried out with a post hoc Bonferroni test to compare the baseline vs. final values, as well as the final values between the group of men and women of all the immunoglobulins evaluated. The data analysis was carried out with the SPSSv23 program and a p < 0.05 was considered as statistical significance. The minimum expected statistical power was 80% for each comparison group.

III.- RESULTS

Twenty-six healthy students were included in this study. 69.2% (18 cases) were women and 30.8% (8 cases) were men. The average age was 20.27±2.2 years with a minimum of 18 and a maximum of 25 years.

No difference was observed in relation to the average age and sex (p>0.05). No statistical difference (p>0.05) was obtained between the average body mass index (BMI) between women (23.49±4.54 Kg/m2) and men (23.21±5.13 Kg/m2). According to the above, all the students participating in this study were classified by their BMI as normal weight.

Figure 1 shows the weight and BMI averages that were determined from the beginning to the end of the study. It can be seen that men had less variability in both anthropometric measurements over time compared to women. No significant differences were observed when evaluating the baseline measurement vs. the final one in men (p>0.05) or in women (p>0.05).

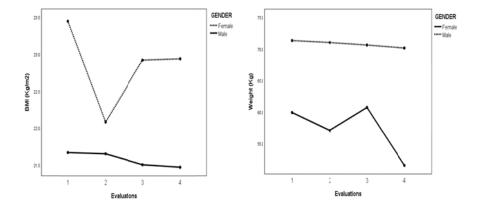


Figure 1. Comparison of the BMI and weight averages in relation to the sex of the study participants that were registered over time

Vol. 4 Issue 3, March - 2022

Figure 2 shows the averages and standard errors of IgA and IgD determined in the 4 measurements and in relation to gender. Regarding IgA, no significant difference was obtained in relation to gender in the final evaluation (p=0.97), nor was there a difference when comparing the baseline vs. final measurement in women in a selfcontrolled manner (412.38±89.66 vs. 765.55±79.11 mg /dl, p=0.09) and in men (450.31±134.49 vs 769.3±118.67 mg/dl, p=0.79). In the case of the IgD analysis, no significant difference was obtained in relation to gender in the final evaluation (p=0.70), neither when comparing the baseline vs final measurement in women in a self-controlled manner (17.12 ± 1.78 vs 11.02 ± 1.44 mg/dl, p=0.02), plus, this was not significant in men (15.63 ± 2.67 vs 10.0 ± 2.16 mg/dl, p=0.39). The statistical power in the case of IgA for the group of women was 84% and for men it was 62% respectively and in the evaluation of IgD the statistical power for women was 79% while for men it was 64%.

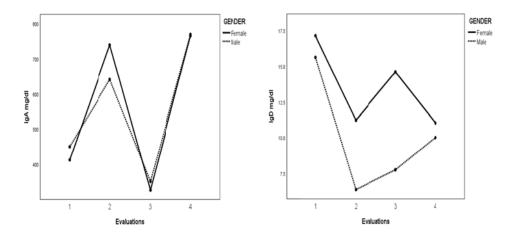
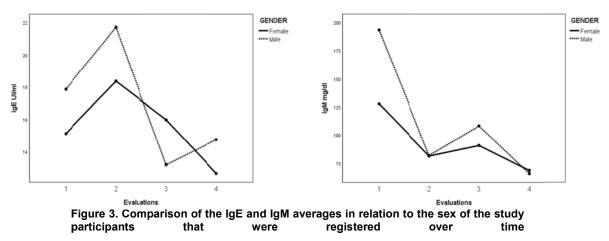




Figure 2. Comparison of IgA and IgD Means of Study Participants Recorded Over

Figure 3 shows the averages and standard errors of IgE and IgM determined in the 4 measurements and in relation to gender. Regarding IgE, no significant difference was obtained in relation to gender in the final evaluation (p=0.52). In the self-controlled analysis of baseline vs. final measurement, no statistically significant differences were observed either in the group of women $(15.11\pm1.94 \text{ vs.} 12.63\pm1.82 \mu \text{I/ml}, p=1.0)$ and in the group of men $(17.86\pm2.9 \text{ vs} 14.74\pm2.73 \mu \text{I/ml}, p=1.0)$. In the case of the IgM analysis, no significant difference was obtained in relation to gender in the final evaluation (p=0.74).



In the self-controlled analysis of baseline vs. final measurement, no statistically significant differences were observed either in the group of women (127.75 ± 47.07 vs. 68.51 ± 4.85 mg/dl, p=1.0) and in the group of men (193.2 ± 70.61 vs 65.67 ± 7.27 mg/dl, p=0.54). The statistical power in the case of IgE for the group of women was 62% and 79% for the group of men. In the IgM evaluation, the

statistical power for women was 71% while for men it was 79%.

Figure 4 shows the averages and standard errors of IgG determined in the 4 measurements and in relation to gender. No significant difference was obtained in relation to gender in the final evaluation (p=0.60), but when comparing the baseline vs final measurement in women in a self-controlled

manner (1181.2 \pm 34.6 vs 1666.9 \pm 124.6 mg/dl, p=0.002) and in men (1266.2 \pm 51.93 vs 0.1785 \pm 186.9 mg/dl, p=0.04).

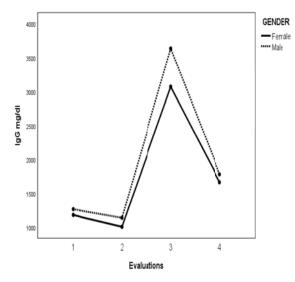


Figure 4. Comparison of the IgG averages in relation to the sex of the study participants that were registered over time

IV.- DISCUSSION

Immunoglobulins are an important component of humoral adaptive immunity, their function is the recognition and neutralization of harmful agents for the body, thus, their analysis reflects information on the individual's health status.

When analyzing the results of the study of immunoglobulins in healthy young people, changes were found in the concentration of IgA in both groups; we can observe an increase above the normal physiological range from the baseline measurement. Normal IgA values range from 99 to 400mg/dl (12,13,14). The concentration of IgA had a stabilization in normal physiological ranges in adjustment number 16 and a greater increase in adjustment number 20. These data are similar to what Ronald found in 1986 where the subjects who received chiropractic care had an immunological competence of 200% higher than those who had not received chiropractic care (11).

IgG is a memory immunoglobulin, its normal values are 768 to 1728 mg/dl, (12,15,16,17) an increase in IgG concentration was observed in spinal adjustment number 16 and a stabilization within normal values was observed in adjustment number 20. These data coincide with that obtained by Teodorczyk in 2010 when they found an increase in the concentration of IgG and IgM within normal physiological ranges after receiving vertebral adjustments (18)

With these data we can conclude that the results obtained in this study are similar to previous studies assessing the effectiveness of spinal adjustment on the immune system.

Like Chow in 2021 in his systematic review (24), no significant clinical evidence is found to support or contradict the efficacy of spinal manipulations on the immune system, but this study shows data suggesting that spinal

The statistical power in the case of IgG for the group of women was 83% and for the group of men it was 78%. manipulation can be associated with changes in biomarkers of the immune system.

On the other hand, the review carried out by Columbi (25) suggests that the mechanical stimulus caused by vertebral adjustment can trigger a cascade of neurophysiological responses orchestrated by coactivation of the autonomic nervous system.

The findings suggest that immunoglobulin concentrations were not constant throughout the study, thus, it would be favorable to design a complementary study on the durability of the adjustment reaction and on people with previously diagnosed pathologies or even in immunocompromised people, plus we suggest increasing the size of the population evaluated as well as the duration of the study.

V.- CONCLUSION

Vertebral manipulations in healthy people generate changes in the concentration of Immunoglobulins within normal physiological ranges since the population is a young and healthy population where the immune system works efficiently. This study gives guidelines to generate new studies on the efficacy of spinal manipulation on the immune system in people with different characteristics (disease), having as background the results on healthy and young people.

VII.- REFERENCES

- Parkin, J., Cohen, B. (2001). An overview of the immune system. *Immunology*, 357 (9270),1777-1789. https://doi.org/10.1016/S0140-6736(00)04904-7
- Ayala, E. (2009). Psiconeuroinmunología. Interrelación entre los sistemas nervioso, endocrino e inmunitario. *Elsevier*, 28 (6), 110-116
- 3. Conh, A. (2008). Chiropractic and the Neuroimmune Connection. *J. Vertebral Subluxation Res.* pp,1-5
- Tiselius, A., Kabat, EA. (1939). An electrophoretic study of inmune sera and purified antibody preparations. *The Journal of experimental Medicine*, 619(1), 119-131. <u>https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2</u> <u>133726/</u>
- Ladrón, E. (2010) Análisis de la interacción anticuerpoheveína mediante espectroscopia de resonancia magnética nuclear. *Tesis de Maestría en Ciencias*, Universidad Nacional Autónoma de México. pp: 3-5
- 6. Mamani, R., Tito, E. (2011). Inmunoglobulinas. *Rev. Act. Clin. Med*, vol. 13,
- Ma, H., O'Kennedy, R. (2015). The Structure of Natural and Recombinant Antibodies. Methods. Mol Biol. 1348: pp. 7-11. https://pubmed.ncbi.nlm.nih.gov/26424258/
- Manohar, MP,. (1992). The stabilizing system of the spine. Part I: Function, Dysfunction, Adaptation, and Enhancement. *Journal of spinal disorders & techniques*. 25 (4), 383-9 https://pubmed.ncbi.nlm.nih.gov/1490034/
- 9. Kuchera, M,. (1995). Gravitational stress, musculoligamantous strain, and postural alignment. Spine, *State of Art Reviews*, 9:463-89.

Vol. 4 Issue 3, March - 2022

- Teodorczyk, J., Injeyan, S., Ruegg, R. (2006). Spinal manipulative therapy reduces inflamatory cytokines but not substance p production in normal subjects. *J Manipulative Physiol Ther*, 29 (1), 14-21. https://pubmed.ncbi.nlm.nih.gov/16396725/
- 11. World Federation of Chiropractic, (2020). The Effect of Spinal Adjustment /Manipulation on Immunity and the Immune System: A Rapid Review of Relevant Literature.
- Saldarriaga, L,. Delgado, L,. Ríos, B,. (2016). Rheumatoid arthritis and thrombocytopenic purpura associated with common variable immunodeficiency, *Editorial Ciencias Médicas*, 18(1)
- Woof, J., Russell, M. (2011). Structure and function relationships in IgA. *Mucosal Immunology*. 4, pp: 590–597. https://pubmed.ncbi.nlm.nih.gov/21937984/
- 14. Leusen, J. (2015). IgA as therapeutic antibody. *Molecular Immunology*, 68 (1): 35-39. <u>https://pubmed.ncbi.nlm.nih.gov/26597204/</u>
- Ma, H., O'Kennedy, R. (2015). The Structure of Natural and Recombinant Antibodies. *Methods Mol Biol.* 1348: pp. 7-11. https://pubmed.ncbi.nlm.nih.gov/26424258/
- Goulet, D., Atkins, W. (2020). Considerations for the Design of Antibody-Based Therapeutics, J Pharm Sci. 109 (1), 74-103
- Yamaguchi, Y., Barb, A. (2020). A synopsis of recent developments defining how N-glycosylation impacts immunoglobulin G structure and function. <u>Glycobiology.</u> 30 (4), 214–225. <u>https://pubmed.ncbi.nlm.nih.gov/31822882/</u>

- Teodorczyk, J., McGregor M, Ruegg R, Injeyan HS. Interleukin 2-regulated in vitro antibody production following a single spinal manipulative treatment in normal subjects. *Chiropr Osteopat.* 2010. 18:26. <u>https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2</u> 945351/
- Arce, A., Villaescusa, R. (2003). Inmunoglobulina D: propiedades y relevancia clínica. *Rev Cubana Hematol Inmunol Hemoter*, 19(2-3)
- 20. Mix, E., Goertsches, R., Zett, U. (2006) Immunoglobulins—Basic considerations. Journal of Neurology, 253, pp: v9–v17. https://pubmed.ncbi.nlm.nih.gov/16998762
- Flegel, W. (2015). Pathogenesis and mechanisms of antibody-mediated hemolysis. *Transfusion*, 55 Suppl 2 (0): S47-58. <u>https://pubmed.ncbi.nlm.nih.gov/26174897/</u>
- Sutton, B., Davies, A. (2015). Structure and dynamics of IgE-Receptor interactions: FceRI and CD23/Fce RII. *Immunol Rev.* 268 (1), 222-35 https://pubmed.ncbi.nlm.nih.gov/26497523/.
- Baillieau, F. (2015). Inmunoglobulina E, revisión y actualización de su rol en la salud y en la enfermedad. Archivos de alergia e inmunología clínica. 46 (2), 55-66
- Chow, N., Hogg-Johnson S, Mior S "et al", (2021). Assessment of Studies Evaluating Spinal Manipulative Therapy and Infectious Disease and Immune System Outcomes: A Systematic
- 25. Review. JAMA Netw Open. 4(4), e 2154. https://pubmed.ncbi.nlm.nih.gov/33847753/
- Columbi, A., Testa, M. (2019). The Effects Induced by Spinal Manipulative Therapy on the Immune and Endocrine Systems. *Medicina (Kaunas)*. 55 (8), 448. https://pubmed.ncbi.nlm.nih.gov/31394861