Secretory Immunoglobulin A and Upper Cervical Chiropractic: A Preliminary Prospective, Multicenter, Observational Study

Philip R. Schalow, DC, DCCJP, [a] Kelly A. Kimball, DC, [b] Frederick T. Schurger, DC, DCCJP, [c] George R. Sooley, DC, DCCJP, [d] Scott P. Bales, DC, DCCJP, [e] Roderic P. Rochester, DC, FCCJP, [f] Robert T. Brooks, DC, [g] and Julie M. Hunt, DC, FCCJP, DICCP [h]

Abstract

Objective: The objective of this study was to observe changes in secretory immunoglobulin A (SIgA) after chiropractic care using upper cervical adjusting techniques (UCATs) at the craniocervical junction (CCJ).
Methods: Forty-one participants were enrolled from 5 chiropractic offices in North America. Each participant provided a saliva sample at an initial visit before care. A second saliva sample was collected after resting 30 minutes after the first UCAT treatment. A third saliva sample was collected after 2 weeks.
Results: There was a significant increase in SIgA observed 30 minutes after the UCAT treatment compared to baseline. After 2 weeks, SIgA levels decreased back to near original levels.
Conclusion: Our preliminary findings demonstrate an immediate, temporary increase in SIgA levels after a UCAT treatment at the CCJ. (J Chiropr Med 2021;20;121-127)
Key Indexing Terms: *Chiropractic; Immunoglobulin A, Secretory; Immunity, Mucosal*

Introduction

Secretory immunoglobulin A (SIgA) is the most abundant immunoglobulin in mucosal secretions.[1] These secretions play a critical role as a part of the immune system, which provides a nonspecific, first-line defense against numerous pathogens.[1] SIgA limits the access of microorganisms to mucosal surfaces in the intestines, respiratory tract, and urogenital tract.[1] It is also a marker for the systemic immune system, as it is produced by B cells and then released into mucosal cells for excretion.[2] Measuring SIgA levels can be an informative indicator of the systemic immune system. SIgA fluctuates with circadian rhythms,[3,4] so collecting samples at a consistent time of day is imperative for making accurate comparisons.

The nervous system is known to regulate the immune system, both directly and hormonally.[5] Both sympathetic and parasympathetic control have been shown to modulate intestinal SIgA[6] as well as salivary SIgA,[7,8] and manual therapy has also been shown to affect SIgA levels.[9] There is a small body of evidence suggesting a relationship between spinal manipulation and the nervous system.[10-13] Some theories of how upper cervical adjusting techniques (UCATs) may influence the nervous system have been proposed.[14] It is hypothesized that spinal manipulation may trigger the neuroimmunoendocrine system,[15] although mechanisms are still being explored.

Therefore, the primary purpose of this study was to observe the influence of the UCAT treatment on SIgA. We hypothesized that there would be a significant increase in SIgA at either or both time points after the UCAT treatment. We also investigated 2 additional biomarkers as secondary outcomes: C-reactive protein (CRP), which has been shown to rise in the acute phase of inflammation as well as in long-term inflammatory processes,[16] and salivary α-amylase, which has been proposed as a biomarker for activation of the sympathetic nervous system.[17] Another secondary outcome was to investigate how responses to the

[a] Private practice, Rockford, Illinois.
[b] International Chiropractors Association, Falls Church, Virginia.
[c] Private practice, Springfield, Illinois.
[d] Private practice, Danville, Illinois.
[e] Private practice, Alliston, Ontario, Canada.
[f] Private practice, Clarkesville, Georgia.
[g] Tulsa, Oklahoma.
[h] Private practice, Clearwater, Florida.
Corresponding author: Kelly A. Kimball.
(e-mail: *k.kimballresearch@gmail.com*).
Paper submitted September 8, 2020; in revised form October 21, 2021; accepted October 21, 2021.
1556-3707

https://doi.org/10.1016/j.jcm.2021.10.003

12-Item Short Form Health Survey version 2 (SF-12v2) might change from baseline to 2 weeks after treatment.

Methods

Upper cervical adjusting techniques (UCATs) are forms of chiropractic manipulation or spinal manipulative therapy that focus on reducing measurable misalignments or dysfunctions at the craniocervical junction (CCJ).[14] We conducted a prospective, multicenter, 2-week pilot study at 5 upper cervical chiropractic practices across North America, each practicing a different UCAT: Atlas Orthogonal, Blair, Knee Chest (Kale/KCUCS), National Upper Cervical Chiropractic Association, and Orthospinology. These techniques were chosen because they were the only UCATs at the time of the study that had both an organization overseeing a certification program in the technique and a doctor of chiropractic in clinical practice with a diplomate or fellowship in chiropractic craniocervical junction procedures (DCCJP, FCCJP) through the International Chiropractors Association's Council on Upper Cervical Care.

Together, the 5 UCATs represent the majority of chiropractors practicing upper cervical chiropractic. Each of the 5 UCATs is represented in the International Chiropractors Association's Council on Upper Cervical Chiropractic Care; uses objective radiographic analysis with a protocol developed and standardized by the respective certifying organization; includes a systematic protocol to determine when and when not to adjust a patient[14]; and has demonstrated the potential for beneficial outcomes over a short period of time.[18]

Five volunteers were recruited from qualified diplomates and fellows. The Sherman College of Chiropractic Institutional Review Board approved the research methods before participant recruitment and data collection. All participants provided consent.

Eligibility criteria

To meet the inclusion criteria, each participant needed to be between 21 and 65 years of age, be a new patient, have clinical findings indicating UCAT treatment, be willing to forego exercise 24 hours before data collection, and be willing to avoid caffeine and alcohol consumption 4 hours before data collection. Potential participants were excluded from the study if they had disease of the salivary glands, had active oral lesions, used tobacco products (cigarette, chewing tobacco, e-cigarettes), used antibiotics or probiotics, had an autoimmune disease, had a respiratory illness within the week before the initial visit, had dental care pending or completed within the week before the initial visit, were currently pregnant, had chiropractic manipulative therapy within the week before the initial visit, were unable to read or comprehend English, or had a contraindication to UCAT treatments.

Recruitment

Participants were recruited from 5 chiropractic practices between January 1, 2019, and June 1, 2019. Each new patient at the 5 clinics during this time frame underwent a consultation, an examination, and imaging to determine whether they were eligible to participate in the study. Patients who met the inclusion criteria were invited to participate, and those who consented were enrolled. Patients who met the exclusion criteria were not invited to participate. Each of the 5 locations was permitted to enroll up to 10 participants within the recruitment time frame. After June 1, 2019, no additional participants were enrolled.

Study design

Each participant signed an informed consent document before data collection. At the first visit, they completed a demographic survey and the SF-12v2. The administration of the SF-12v2 followed all guidelines outlined in the user's manual.[19] Salivary samples were collected immediately before the first treatment, after the first UCAT treatment at the CCJ after a 30-minute rest, and at the end of the 2-week period. The UCAT performed was Atlas Orthogonal, Blair, Knee Chest, National Upper Cervical Chiropractic Association (NUCCA), or Orthospinology, depending on which doctor the participant saw, because each doctor in the study practiced a different UCAT. Each UCAT has its own standardized protocol,[14] which was strictly followed by each doctor. SIgA levels demonstrate circadian variation,[3,4] so each follow-up sample was collected within a 2-hour range centered on the time of the initial sample. For example, if the participant's initial sample was collected at 10 AM, each follow-up sample was collected within 1 hour before or after 10 AM. There were 5 visits for each participant within a 2-week period, including the initial visit. At each visit, the investigator examined the participant to ascertain whether or not an additional treatment was required, following the protocols designated by the respective UCAT. Each participant completed the SF-12v2 again at the end of the 2-week period.

All clinical data collected related to this project were stored on paper documents. These documents included the informed consent, inclusion/exclusion checklist, SF-12v2, and daily-visit checklists, which indicated the procedures performed at each visit. These were stored in a secure file until the completion of the project, at which time they were copied and sent to the statistician for processing. Each

document was identifiable only by the same alphanumeric identifier that was associated with that participant's saliva sample, so that the statistician was blinded to the identity of any participant.

Salivary-sample processing

Saliva was collected according to Salimetrics' lab instructions and marked with an alphanumeric designation indicating the participant and the number of the sample. The specimen was immediately stored in the office refrigerator and then deep-frozen to 0°F. At the completion of sample collection for the office, all samples were shipped in dry ice to the lab. Samples were assayed at the Salimetrics SalivaLab (Carlsbad, California) using the Salimetrics Salivary Secretory IgA ELISA Kit (catalog number 1-1602), without modifications to the manufacturer's protocol. Salimetrics has demonstrated no significant difference between its protocol and a commercially available SIgA enzyme-linked immunosorbent assay for determining SIgA levels.[20]

Statistical analysis

The software used for statistical analysis was IBM SPSS Statistics 26. Mean levels of SIgA, CRP, and α-amylase were compared at baseline, 30 minutes after the first treatment, and after 2 weeks using a repeated-measures analysis of variance with $\alpha = 0.05$. Data were transformed before analysis so that normality and variance assumptions were met. We used the Shapiro-Wilk test for normality. Pairwise comparisons were accomplished using a Bonferroni correction.

The SF-12v2 was scored following all guidelines detailed in the user's manual.[19] Participant responses were entered manually into Excel and scored using the computer program PRO CoRE version 1.4.[21] The program scores each survey compared to a standardized baseline based on US adults, which is set to 50. Scores range from 0 to 100, with 100 indicating better health status. The SF-12v2 measures health status in both physical health and mental health, reporting a physical component score (PCS) and a mental component score (MCS). A Wilcoxon signed-rank test was performed to evaluate whether the PCS and MCS were significantly different at the 2-week time point compared to their respective baselines.

Results

A total of 358 new patients were considered for participation in this study. Of those, 116 met the inclusion and not the exclusion criteria, and 44 of these consented to participate. Three participants were lost to follow-up after the initial visit, giving a total of 41 participants who were included in the data analysis.

Demographic information

The demographic information about the study participants is summarized in Table 1. Our study participants were 56.1% women, and most respondents were white, non-Hispanic, married, and employed. The age of participants ranged from 25 to 65 years, with a mean of 47.5 years and a median of 49.5 years.

Secretory IgA

A repeated-measures analysis of variance demonstrated a significant difference in mean SIgA among our 3 time points, $F_{2,78} = 9.63$, $P < .01$. Post hoc analysis was accomplished using a Bonferroni correction. A significant increase of 117.85 μg/mL, $P < .01$, was observed in mean SIgA level from baseline (mean [M] = 311.05, SD = 202.37) to posttreatment (M = 428.90, SD = 329.70), as represented in Figure 1. There was also a significant decrease in mean SIgA observed after 2 weeks (M = 284.45, SD = 174.48) compared to the posttreatment level, $P < .01$. There was no significant difference between the mean SIgA level initially and after 2 weeks, $P > .017$.

C-reactive protein and α-amylase

No statistical difference was observed in mean CRP at baseline, after treatment, or at 2 weeks, $F_{2,64} = 0.973$, $P = .384$. There was also no observed difference in mean α-amylase among the 3 time points, $F_{1,37} = 1.294$, $P = .263$.

12-Item Short Form Health Survey Version 2

The mean PCS increased significantly from baseline (M = 45.0, SD = 10.75) to 2 weeks (M = 48.3, SD = 9.15), $Z = 524.5$, $P = .025$. The mean MCS also increased significantly from baseline (M = 50.1, SD = 10.17) to 2 weeks (M = 52.3, SD = 7.70), $Z = 381.5$, $P = .028$.

Discussion

To our knowledge, this study is the first investigation to measure SIgA after UCAT treatment. Although human studies exploring SIgA levels have been completed in cross-sectional samples,[9,22-24] this study is among the first to investigate SIgA samples longitudinally in human subjects.

We measured an increase in mean SIgA 30 minutes after the first treatment, compared to both the initial sample and the sample taken after 2 weeks. However, there was no significant change between the initial and final samples, though the mean value after 2 weeks was lower than the initial value. Our findings are similar to those observed in individuals who received osteopathic manipulative therapy,

Table 1. *Demographic Information for Study Participants (n = 41)*

Characteristic	n	%
Sex		
Female	23	56.1
Male	18	43.9
Age, y		
25-29	6	14.6
30-34	2	4.9
35-39	4	9.8
40-44	3	7.3
45-49	5	12.2
50-54	7	17.1
55-59	8	19.5
60-64	5	12.2
65-69	1	2.4
Race		
American Indian or Alaska Native	0	0
Asian	1	2.4
Black or African American	1	2.4
Native Hawaiian or Other Pacific Islander	0	0
White or Caucasian	38	92.7
Multiple races	1	2.4
Ethnicity		
Hispanic	1	2.4
Non-Hispanic	34	82.9
Unknown or not reported	6	14.6
Marital status		
Single (never married)	10	24.4
Married	24	58.5
Divorced	5	12.2
Widowed	1	2.4
Living with significant other	1	2.4
Education		
Some high school	0	0
High school diploma	10	24.4
Some college	5	12.2
College degree	12	29.3
Postgraduate degree	2	4.9
Trade school	5	12.2
Professional school	0	0
Not reported	7	17.1
Employment		
Self-employed	7	17.1
Employed by other	28	68.3
Work in home	1	2.4
Unemployed	1	2.4
Student	0	0
Retired (not ill health)	2	4.9
Disabled/retired (ill health)	0	0
Not reported	2	4.9

who also showed a significant increase in SIgA after treatment.[9] While our mean level of SIgA at baseline was nearly 3 times higher than in the osteopathic study—possibly due to that study's highly stressed participant population, who might have lower SIgA levels as a result of stress—our mean increase of 117.85 μg/mL (n = 40) was similar to their observed 139-mg/L (n = 12) increase.[9] The osteopathic study also had a control group, which rested for 20 minutes instead of receiving osteopathic manipulative therapy, that showed a modest 35-mg/L (n = 13) increase in SIgA. We recommend using a similar control group in future research on the relationship between SIgA and chiropractic treatments. Our findings also showed no significant difference in the mean level of SIgA between men and women at any of the time points, similar to other studies.[25]

As SIgA is thought to be a marker for the status of not only the mucosal immune system[26] but also systemic immunity,[2] an increase in SIgA may indicate activation of the systemic immune system. Our findings suggest that

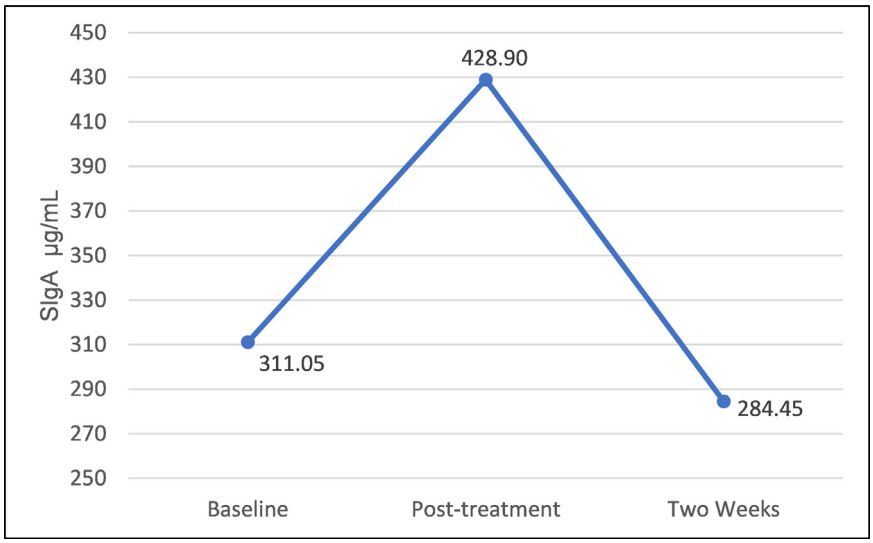

Fig. 1. *Mean secretory immunoglobulin A before, 30 minutes after, and 2 weeks after an initial upper cervical adjusting technique treatment at the craniocervical junction (n = 40).*

after a UCAT treatment at the CCJ, a systemic immune response is activated for a short period.

Mechanisms to explain our findings are not entirely clear. Chiropractic care may alleviate physiological stress in the body as well as self-perceived emotional stress,[27] which may contribute to these SIgA findings. Several studies have shown that SIgA is sensitive to physiological and emotional stress. For example, perceived stress, loneliness, and depressive symptoms have all been shown to decrease various subclasses of SIgA in undergraduate students.[23] Emergency department nurses have reported higher stress levels and lower SIgA secretion than general ward nurses.[22] Additionally, abbreviated progressive muscle relaxation has been shown to increase SIgA levels in undergraduate students.[24] The relationship between stress and SIgA secretion has been replicated in animal studies, where SIgA has been shown to decrease in both physiologically and psychologically stressed rodents.[6] Future research could explore how loneliness, depressive symptoms, and emotional stress may contribute to SIgA responses in chiropractic patients by using outcome measures such as the Revised UCLA Loneliness Scale, the Beck Depression Inventory, and the Perceived Stress Scale, as well as investigating how these outcome measures may change throughout a course of chiropractic care.

SIgA may be sensitive to neurological changes. Animal studies have shown that there is a link between the autonomic nervous system and SIgA levels. For example, SIgA has been shown to decrease when parasympathetic input is removed from the submandibular gland in rats.[28] It has also been shown in animal models to increase when stimulated by norepinephrine.[3] While the relationship between the immune system and the sympathetic nervous system has been studied in depth,[29] further research is necessary to better understand the role the autonomic nervous system plays in SIgA secretion specifically, as well as the neuroimmunoendocrine effect of UCAT treatments at the CCJ. Heart-rate variability is a useful indicator of the autonomic nervous system and has been shown to change as the result of chiropractic spinal manipulation.[10,12,30] Future research could investigate how heart-rate variability and SIgA levels change before and after chiropractic care.

Lee proposed a thalamic neuron theory[31] that may be a plausible explanation for our findings, which is that the immune system is modulated by the central nervous system. Lee proposed that the nervous system, immune system, and endocrine system have an inseparable relationship, and noted that boundaries between them are "both anatomically and molecularly blurred."[31] He proposed that the immune system could be considered a component of the central nervous system. Further research is necessary to explore the mechanisms behind our observations regarding SIgA secretion, as well as the relationship between UCAT treatments and immune function.

We found improvement in both the PCS and MCS of the SF-12v2 from baseline to 2 weeks. While the SF-12v2 is more commonly used cross-sectionally in the chiropractic literature, our study examined how it changed longitudinally in participants under chiropractic care. We recommend that future research continue to administer surveys past 2 weeks to investigate how physical and mental health scores may change over time, as measured by the SF-12v2.

Our findings did not show a significant change in α-amylase during our 2-week study; however, we did observe that it was decreased at 2 weeks compared to the level observed after treatment. We recommend that future

studies continue collecting samples past 2 weeks to see if this trend develops. We also did not observe a significant change in CRP throughout the study, because CRP levels remained fairly consistent at the 3 time points.

Limitations

Our data demonstrated large standard deviations, which may be the result of our small sample size. In the statistical analysis of SIgA, 1 data point was removed as an outlier for exhibiting very high SIgA in the posttreatment sample. Removing this data point did not change the significance of our findings, and it improved the variability of our data set. Eight of the study participants had 1 or more missing CRP data points because they had CRP samples above the upper limit of 8000 pg/mL or because there was an insufficient amount of sample to be processed, making them unable to be analyzed by the Salimetrics SalivaLab. As a result, these 8 individuals were omitted from the analysis of variance for CRP. There were also 3 study participants who did not complete the SF-12v2 at the 2-week time point. These individuals were omitted from the statistical analysis of the SF-12v2 health surveys.

The generalizability of our findings is limited by our small sample size and short follow-up length of 2 weeks. Because this is a small study, we recommend replicating it on a larger scale, increasing both the number of participants and the length of time following them, to explore how SIgA, CRP, and α-amylase might change over a period of time longer than 2 weeks. We also recommend collecting saliva samples more frequently than 2 weeks apart, because this could provide more information on how SIgA responds after treatment.

The generalizability of our findings is also limited to the 5 UCATs included, performed by chiropractors who were upper cervical diplomates and fellows. For future studies, we recommend using a larger and random sample of upper cervical diplomates and fellows. Sources of error were minimized by standardizing each patient visit, because each doctor strictly adhered to the procedures outlined by their specific UCAT. Further research into the neuroimmunoendocrine effects of chiropractic care is encouraged to investigate the underlying mechanisms of these findings.

And finally, although there was a statistically significant increase in SIgA laboratory findings, we measured the clinical effects of this increase using only the SF-12v2. A more robust list of clinical outcomes should be measured before clinical or treatment recommendations can be made. This serves as a preliminary study for further research into the relationship between UCAT treatments and immune function. We recommend that future studies test how the application of UCAT treatments may influence a person's immune system in both the short and long term.

Conclusions

Our findings demonstrated an increase in SIgA levels from baseline to 30 minutes after a UCAT treatment at the CCJ. We also found that there was an increase in both the PCS and MCS of the SF-12v2 from baseline to 2 weeks. No significant change was observed in CRP or α-amylase at any of the time points, although α-amylase demonstrated a decreasing trend over the 2-week period.

Practical Applications

- Our study is the first to investigate the relationship between secretory immunoglobulin A and upper cervical adjusting technique treatments at the craniocervical junction.
- A temporary increase in secretory immunoglobulin A was observed immediately after an upper cervical adjusting technique treatment at the craniocervical junction.
- Our study is among the first to explore secretory immunoglobulin A samples longitudinally in human subjects.

Acknowledgements

We acknowledge Beth Clay for her thoughtful review of our manuscript.

Funding Sources and Conflicts of Interest

This project was financially supported by the Orthospinology Research Fund and Upper Cervical Research Foundation. Our funding sources had no role in the design or the collection, analysis, or interpretation of the data. Optum does not endorse this paper. No other conflicts of interest were reported for this study.

Contributorship Information

Concept development (provided idea for the research): P.R.S., F.T.S., S.P.B., R.P.R., J.M.H.
Design (planned the methods to generate the results): P.R.S., F.T.S., G.R.S., R.P.R., J.M.H.
Supervision (provided oversight, responsible for organization and implementation, writing of the manuscript): P.R.S., F.T.S., R.T.B., J.M.H.
Data collection/processing (responsible for experiments, patient management, organization, or reporting data): P.R.S., F.T.S., G.R.S., S.P.B., J.M.H.

Analysis/interpretation (responsible for statistical analysis, evaluation, and presentation of the results): P.R.S., R.P.R.
Literature search (performed the literature search): P.R.S., K.A.K.
Writing (responsible for writing a substantive part of the manuscript): P.R.S., K.A.K.
Critical review (revised manuscript for intellectual content, this does not relate to spelling and grammar checking): P.R.S., K.A.K., S.P.B., R.P.R.

References

1. Corthésy B. Multi-faceted functions of secretory IgA at mucosal surfaces. *Front Immunol*. 2013;4:185.
2. Brandtzaeg P. Do salivary antibodies reliably reflect both mucosal and systemic immunity? *Ann N Y Acad Sci*. 2007;1098:288-311.
3. Wada M, Orihara K, Kamagata M, et al. Circadian clock-dependent increase in salivary IgA secretion modulated by sympathetic receptor activation in mice. *Sci Rep*. 2017;7 (1):8802.
4. Li T-L, Gleeson M. The effect of single and repeated bouts of prolonged cycling and circadian variation on saliva flow rate, immunoglobulin A and alpha-amylase responses. *J Sports Sci*. 2004;22(11-12):1015-1024.
5. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat Rev Immunol*. 2006;6(4):318-328.
6. Campos-Rodríguez R, Godínez-Victoria M, Abarca-Rojano E, et al. Stress modulates intestinal secretory immunoglobulin A. *Front Integr Neurosci*. 2013;7:86.
7. Bishop NC, Gleeson M. Acute and chronic effects of exercise on markers of mucosal immunity. *Front Biosci*. 2009;14:4444-4456.
8. Tsujita S, Morimoto K. Secretory IgA in saliva can be a useful stress marker. *Environ Health Prev Med*. 1999;4(1):1-8.
9. Saggio G, Docimo S, Pilc J, Norton J, Gilliar W. Impact of osteopathic manipulative treatment on secretory immunoglobulin a levels in a stressed population. *J Am Osteopath Assoc*. 2011;111(3):143-147.
10. Wirth B, Gassner A, de Bruin ED, et al. Neurophysiological effects of high velocity and low amplitude spinal manipulation in symptomatic and asymptomatic humans: a systematic literature review. *Spine (Phila Pa 1976)*. 2019;44(15):E914-E926.
11. Picchiottino M, Leboeuf-Yde C, Gagey O, Hallman DM. The acute effects of joint manipulative techniques on markers of autonomic nervous system activity: a systematic review and meta-analysis of randomized sham-controlled trials. *Chiropr Man Therap*. 2019;27:17.
12. Welch A, Boone R. Sympathetic and parasympathetic responses to specific diversified adjustments to chiropractic vertebral subluxations of the cervical and thoracic spine. *J Chiropr Med*. 2008;7(3):86-93.
13. Pickar JG. Neurophysiological effects of spinal manipulation. *Spine J*. 2002;2(5):357-371.
14. Woodfield 3rd HC, C York, Rochester RP, et al. Craniocervical chiropractic procedures—a précis of upper cervical chiropractic. *J Can Chiropr Assoc*. 2015;59(2):173-192.
15. Colombi A, Testa M. The effects induced by spinal manipulative therapy on the immune and endocrine systems. *Medicina*. 2019;55(8):448.
16. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. *Front Immunol*. 2018;9:754.
17. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology*. 2009;34 (4):486-496.
18. Eriksen K, Rochester RP, Hurwitz EL. Symptomatic reactions, clinical outcomes and patient satisfaction associated with upper cervical chiropractic care: a prospective, multicenter, cohort study. *BMC Musculoskelet Disord*. 2011;12:219.
19. Maruish ME. *User's Manual for the SF-12v2 Health Survey*. 3rd ed. Lincoln, RI: QualityMetric; 2012.
20. Salimetrics. Salivary secretory IgA: indirect enzyme immunoassay kit. Available at: https://salimetrics.com/wp-content/uploads/2018/03/secretory-iga-saliva-elisa-kit.pdf. Accessed December 1, 2019.
21. Pro CoRE computer program. Johnston RI. OptumInsight Life Sciences, Inc. 2018.
22. Yang Y, Koh D, Ng V, et al. Self perceived work related stress and the relation with salivary IgA and lysozyme among emergency department nurses. *Occup Environ Med*. 2002;59 (12):836-841.
23. Engeland CG, Hugo FN, Hilgert JB, et al. Psychological distress and salivary secretory immunity. *Brain Behav Immun*. 2016;52:11-17.
24. Pawlow LA, Jones GE. The impact of abbreviated progressive muscle relaxation on salivary cortisol and salivary immunoglobulin A (sIgA). *Appl Psychophysiol Biofeedback*. 2005;30(4):375-387.
25. Rutherfurd-Markwick K, Starck C, Dulson DK, Ali A. Salivary diagnostic markers in males and females during rest and exercise. *J Int Soc Sports Nutr*. 2017;14:27.
26. Mestecky J. Saliva as a manifestation of the common mucosal immune system. *Ann N Y Acad Sci*. 1993;694:184-194.
27. Rhee TG, Marottoli RA, Van Ness PH, Tinetti ME. Patterns and perceived benefits of utilizing seven major complementary health approaches in U.S. older adults. *J Gerontol A Biol Sci Med Sci*. 2018;73(8):1119-1124.
28. Carpenter GH, Proctor GB, Garrett JR. Preganglionic parasympathectomy decreases salivary SIgA secretion rates from the rat submandibular gland. *J Neuroimmunol*. 2005;160(1-2):4-11.
29. Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. *Pharmacol Rev*. 2000;52(4):595-638.
30. Roy RA, Boucher JP, Comtois AS. Heart rate variability modulation after manipulation in pain-free patients vs patients in pain. *J Manipulative Physiol Ther*. 2009;32(4):277-286.
31. Lee TN. Thalamic neuron theory: theoretical basis for the role played by the central nervous system (CNS) in the causes and cures of all diseases. *Med Hypotheses*. 1994;43(5):285-302.