ORIGINAL RESEARCH

Surrogate Indication of DNA Repair in Serum After Long Term Chiropractic Intervention – A Retrospective Study

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ABSTRACT

Objective: To assess the effects of short-term and long-term chiropractic care on serum thiol levels in asymptomatic subjects.

Summary of background data: Serum thiol is a measure of human health status. It is a surrogate estimate of DNA repair enzyme activity, most notably poly ADP–ribose polymerase or PARP. While it is suggested that chiropractic care improves general health, the effect of chiropractic care on serum thiol levels has not been investigated.

Methods: A case controlled retrospective analysis. Serum thiol levels in patients with active disease (N=46) were compared with serum thiol levels in primary wellness subjects with 8-52 weeks of chiropractic care (N=21) and those who had been under chiropractic care for 52-312 weeks (N=25). Patients were age matched to be 40 years of age or older.

Results: There were statistically significant differences in the serum thiol levels of the three groups. Mean serum thiol levels were lowest in patients with active disease as well as patients with initial musculoskeletal complaints. Asymptomatic subjects under chiropractic care demonstrated higher mean serum thiol levels than patients with active disease. Mean serum thiol levels were highest in the group with 52-312 weeks of chiropractic care.

Conclusion: Asymptomatic or primary wellness subjects under chiropractic care demonstrated higher mean serum thiol levels than patients with active disease and produced some values that were higher than normal wellness values.

Keywords: chiropractic, wellness, adjustment, thiol, DNA repair, oxidative stress

Introduction

Alternative and complementary health care

Eisenberg et al.1 reported in 1993 that 34% of adults in the United States used at least one form of "unconventional" health care. Unconventional care was defined as those practices "neither taught widely in U.S. medical schools nor generally available in U.S. hospitals." Extrapolation of the survey indicated that the number of visits to alternative providers exceeded the number of visits to all U.S. primary care physicians.

A follow-up study2 published in 2001 reported 67.6% of respondents had used at least one CAM (complementary and alternative) method in their lifetime. Lifetime use steadily in-

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increased with age across three age cohorts: approximately 3 of every 10 in the pre-baby boom cohort, 5 of 10 in the baby boom cohort, and 7 of 10 in the post-baby boom cohort reported using some form of CAM by age 33 years.

Chiropractic care

Chiropractic is the best established of the alternative health care professions, and it is now more than a century old. Chiropractic is a licensed healthcare profession in the United States, Canada and over 60 other countries worldwide.2,3

According to the Association of Chiropractic Colleges4 "Chiropractic is concerned with the preservation and restoration of health, and focuses particular attention on the subluxation. A subluxation is a complex of functional and/or structural and/or pathological articular changes that compromise neural integrity and may influence organ system function and general health.

A subluxation is evaluated, diagnosed, and managed through the use of chiropractic procedures based on the best available rational and empirical evidence." Most patients present to chiropractors with lower back pain, neck pain, whiplash and headaches,5 which has probably been
reinforced by the fact that of 223 professional organizations surveyed, many reported that these health conditions could be effectively addressed with chiropractic care. What drives the use and popularity of chiropractic is patient satisfaction, and not its endorsement from the scientific or medical professions. In fact, the chiropractic theory that chiropractic care improves specific health conditions remains controversial, principally because of limited scientific validation.

The World Health Organization defines health as being "a state of complete physical, mental, and social well-being, and not merely the absence of disease or infirmity." Given this broad definition of health, epistemological constructs borrowed from the social sciences may demonstrate health benefits not disclosed by randomized clinical trials. Health benefits such as improvement in self-reported quality-of-life, decreased health care costs, behaviors associated with decreased morbidity, and patient satisfaction may be evaluated using such methods.

Blanks, Schuster and Dobson published the results of a retrospective assessment of subluxation-based chiropractic care on self-rated health, wellness and quality of life. This is, to our knowledge, the largest study of its kind ever undertaken regarding a chiropractic population. After surveying 2,818 respondents in 156 practices, a strong connection was found between persons receiving Network care and self-reported improvement in health, wellness and quality-of-life. 95% of respondents reported that their expectations had been met, and 99% wished to continue care.

Coulter et al performed an analysis of an insurance database; comparing persons receiving chiropractic care with non-chiropractic patients. The study consisted of senior citizens over 75 years of age. It was reported that the persons receiving chiropractic care reported better overall health, spent fewer days in hospitals and nursing homes, used fewer prescription drugs, and were more active than the non-chiropractic patients.

Rupert, Manello, and Sandefur surveyed 311 chiropractic patients, aged 65 years and older, who received "maintenance care" for 5 years or longer. Chiropractic patients receiving maintenance care, when compared with US citizens of the same age, spent only 31% of the national average for health care services. There was a 50% reduction in medical provider visits. The health habits of patients receiving maintenance care were better overall than the general population, including decreased use of cigarettes and decreased use of nonprescription drugs. Furthermore, 95.8% believed the care to be either "considerably" or "extremely" valuable.

Rupert reports that 79% of chiropractic patients have maintenance care recommended to them, and nearly half of those comply. In an online survey with 3018 respondents by Miller, 62% responded affirmatively when asked, "Although you feel healthy, would you follow your family member's lead and visit a doctor who focuses on wellness and prevention just so you can stay feeling that way?"

There is therefore a growing body of evidence that wellness care provided by doctors of chiropractic may reduce health care costs, improve health behaviors, and improve patient perceived quality-of-life. Clearly, wellness is well established as a recommended concept for patients. In this context, clinical chemistry has not been utilized by the profession at large, since a symp-

tom or infection is needed to indicate such testing. In short, there is no real point to using a disease test for assessing wellness. The serum thiols test, being an estimate of human health status, however, falls into a clinical category that does not require an infection or symptom before it is employed. As such, it potentially could serve to quantify the wellness levels of Chiropractic patients if a relationship between long term care and thiol value levels can be established.

**Oxidative Stress and DNA repair**

Oxidative stress, metabolically generating free radicals and damaging DNA, is now a very broadly accepted theory of how we age and acquire diseases. A primary source of oxidative stress factors is the central nervous system (CNS), via both neurological and psychological mechanisms.

Because patient satisfaction with chiropractic is high, and yet the biological mechanisms associated with favourable clinical responses still remain unclear, we have sought to study this apparent anomaly by investigating if chiropractic might not inhibit or at least reduce the body’s endogenous production of oxidative stress and the health consequences thereof. Endogenous processes are generally considered to be the most damaging forms of DNA damage.

Oxidative stress not only induces DNA damage but it also inhibits DNA repair, which is the metabolic mechanism that protects genes from becoming damaged by removing the lesions in DNA that have occurred from environmental impact. DNA repair can be estimated in human peripheral blood cells and more recently in a surrogate manner in plasma or serum. Plasma/serum can be used as a surrogate estimate of DNA repair because poly ADP-ribose polymerase (PARP) is a key enzyme that is functionally dependent on redox balance because of cysteine residues in its DNA binding zinc finger domain. Hence, if plasma/serum thiols are quantified they predict the redox state of PARP, and as a consequence, an individual’s DNA repair capacity. Serum thiols are an estimate of mortality (low value), longevity (high value) and presence of active disease (reduced values). It has recently been employed commercially as measure of nutritional intervention, cancer assessment and anti-aging status. It has therefore been theorized that it could be an evaluative technique to measure both conventional and non-conventional clinical interventions.

Here we report on the application of the serum thiol test to patients receiving chiropractic care. The data have indicated a positive influence of chiropractic care by reducing oxidative stress estimated by serum thiols analyses, a surrogate measurement of DNA repair enhancement.

**Materials and Methods**

**Chiropractic Patients.** A case controlled retrospective study was conducted with age and sex matched characteristics experiencing short and long term chiropractic care. All subjects (n = 46) were 40 years of age or older, and all were apparently disease free and had initially come in because of musculoskeletal complaints. At the time of this study the long-term patients essentially had opted for the wellness approach. Short-term care was defined as those subjects (n = 21) who had been under chiropractic care for 8-52 weeks. Long-term care subjects were
defined as those individuals being under regular chiropractic care for 52-312 weeks (n = 25).

Office visit frequencies were determined by the chiropractor, as were clinical procedures, which varied according to spinal adjusting technique, patient response and compliance.

Chiropractic patient sampling was conducted on a randomized basis from patients having short and long term care recruited from two chiropractic clinics; namely Dr. Noel Aboud of Solon, Ohio and Dr. Dennis Ehren of Lakewood, Ohio. Dr. Clayton Campbell, the study design director, obtained informed consents and performed the randomization. After randomization, subjects were instructed to have a heparinized blood sample drawn for thiol testing at Biomedical Diagnostic Research, LLC reference labs (Chesterland Ohio). Plasma was harvested by centrifugation at 600 x g from the blood samples and then stored at −20 C until used for analysis. The blood sampling was completed over a two-month period.

Chiropractic techniques.

The chiropractors involved in this study generally employed one of two methods of chiropractic adjustment:

1. Network Spinal Analysis (NSA). Epstein,28 the developer of NSA stated, "The clinical practice of NSA involves a specific system of classifying vertebral subluxation, which was originally developed in 1982, and described as Network Chiropractic...The system of vertebral subluxation classification and Phasing System is referred to as the Network Protocol...The objective of NSA is to assess and correct two classes of vertebral subluxation; facilitated subluxation and structural subluxation, using safe ‘hands-on’ low-force adjustments of the spine and its contiguous structures." Epstein’s method is described in detail in reference 27.

2. Diversified Technique With Drop-Table. Diversified technique refers to a collection of high-velocity, low amplitude manual techniques.26,27 These techniques involve manual procedures, which take the joint beyond its physiological range of movement, without exceeding the boundaries of anatomical integrity. A "dynamic thrust" is applied, resulting in a gapping of the joint, often accompanied by an audible release. Drop-table techniques involve the use of a special table, where the cushions move a short distance upon application of the dynamic thrust.28,29

3. Activator Methods™. This technique employs the use of a multi-spring loaded, hand held device to perform the spinal adjustment. This instrument generates a local reflex muscular response different from manual technique particularly when the origin or insertion of the muscle is at the level where the vertebra is adjusted.30

Plasma/serum thiol analysis. The thiols present in serum proteins precipitated with 80% saturated ammonium sulfate were determined as previously described with only minor modifications to accommodate a micro scale analysis30. Briefly, 50 ml aliquots of serum were precipitated with 100 ml saturated ammonium sulfate. The resulting suspensions were vortexed, incubated for 15 minutes at room temperature, and the precipitated protein harvested by centrifugation at 1,000 x G. The supernatant was discarded and the pellet was redissolved in 150 ml physiologic saline for 30 minutes. Three 50 ml aliquots of the dissolved protein pellet were prepared as follows: 50 ml sample + 200 ml physiologic saline, 50 ml sample + 200 ml DTNB reagent (5,5'-dithio-bis(2-nitrobenzoic acid), 50 ml physiologic saline + 200 ml DTNB reagent. The DTNB reagent contains 9.5 mg/ml in 0.1M K2HPO4, 17.5 mM EDTA adjusted to pH = 7.5 and diluted 1:50 with water before use. The assay was carried out in 96-well microtiter plates and the absorbance read at 412 nanometers. Subtract saline blank absorbance and DTNB blank absorbance from the absorbance of saline + DTNB + sample. The 80% ammonium sulfate precipitated serum thiol samples were estimated as nmole/l cysteine per 200 ml aliquot of serum using a 0-100 nM cysteine standard curve. The serum samples were assayed in duplicate.

Statistics. Individual values for the patients who had received chiropractic care were analyzed by groups of time under chiropractic care; i.e. 8-52 weeks and 52-312 weeks and compared by t-test statistics. Linear regression analyses associating plasma thiols to weeks of chiropractic care were also performed.

Results

Analyses by t-test comparison to controls. Short term (2-104 weeks, 22 ± 24) and long term (105-868 weeks, 356 ± 216) chiropractic care, who were originally treated at two chiropractic clinics for musculoskeletal complaints, were compared to each other as well as to a non-chiropractic treated control group. The biochemical intermediate endpoint was plasma thiol status in the 80% ammonium sulfate precipitated protein fraction. Plasma/serum thiol status has already been correlated to life span, HIV survival from HIV infection and antioxidant nutritional intervention, indicating this biomarker might have strong potential to detect endogenous oxidative stress levels occurring in vivo that in turn is considered a major risk factor for many human disorders.32,24,25 Hence, it was hypothesized that abnormal transport of neurological pulse signal pathways (a form of dyspnoea) to peripheral tissues might be a major source of generation of endogenous oxidative stress that could be overcome by chiropractic care. The data supporting such a hypothesis are presented in Table 1. It is quite clear that the various groups assembled for analysis were well randomized because there were no statistical differences in either age or sex. Because age and sex have high potential to co-vary with plasma thiol levels as oxidative stress factors, then these data strongly indicate that any differences in plasma thiol status were not influenced or explained by these co-factors.

The average short term chiropractic care group of 22 ± 24 weeks was statistically lower in plasma thiols than the control group, suggesting musculoskeletal complaints indeed could have induced an oxidative stress that was not reversed by short term care. However, long term chiropractic care of 356 ± 216 weeks did significantly reverse the plasma thiol level to be in the normal range or even higher (i.e. from 105 to 146 nM cysteine, Table 1). Taken together it was concluded that musculoskeletal stress discomfort, associated with vertebral subluxation, could induce an in vivo oxidative stress effect estimated by reduced thiol levels in plasma, but it could also be reversed by long term chiropractic care.

Effect of duration of chiropractic care by linear regression. This study had a very wide range of chiropractic care from 2 to 868 weeks of regular visits to the clinic for spinal adjust-
Table 1. Age, sex and plasma thiol levels in patients receiving chiropractic care compared to non-symptom-
atic, apparently healthy controls.

<table>
<thead>
<tr>
<th>Chiropractic care</th>
<th>Age (yr)</th>
<th>Sex (F/M)</th>
<th>Plasma thiols (nM cysteine)</th>
<th>Non-paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Weeks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. None</td>
<td>30</td>
<td>52 ± 10c</td>
<td>17/13c</td>
<td></td>
</tr>
<tr>
<td>(controls)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. 22 ± 24</td>
<td>25</td>
<td>55 ± 11c</td>
<td>16/9c</td>
<td>* = p &lt; 0.003</td>
</tr>
<tr>
<td>3. 356 ± 216</td>
<td>22</td>
<td>50 ± 8c</td>
<td>15/7c</td>
<td>* = p &lt; 0.001</td>
</tr>
<tr>
<td>4. Groups 2 + 3</td>
<td>47</td>
<td>53 ± 10c</td>
<td>31/17c</td>
<td>* = n.s.</td>
</tr>
<tr>
<td>(180 ± 224)</td>
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F/M = female/male; Plasma thiols = nanomolar plasma protein cysteine residues precipitated with 80% ammonium sulfate in 200 ml plasma; n.s. = not significant, sex was analyzed by chi square

ments. Hence, the question of how much care would be sufficient to reverse the plasma thiol reduction seemed appropriate to estimate statistically. For this purpose, we have performed a linear regression analysis of plasma protein thiol levels and the log in weeks of chiropractic care, which was used to better statistically analyze the broad range of duration of treatment values (Fig. 1). There was a statistical significance (p < 0.02) to the positive slope increase in plasma thiols with duration of chiropractic care, which in turn strongly supports the results of t-testing reported in Table 1, when short and long term care were compared as groups. Second, if we estimate from the linear regression line plotted in Fig. 1, how much chiropractic care it would take to reduce the plasma thiols from an average of 105 nM cysteine to control average levels of 127, then the statistical estimate would be about 175 weeks. In summation, these data indicate that whereas chiropractic care can help overcome oxidative stress induced by musculoskeletal stress discomfort, it takes over two years of regular chiropractic treatments to accomplish successful reversal to control levels.

![Figure 1](image)

**Figure 1.** Linear regression analysis of nM cysteine residues present in 80% ammonium sulfate precipitated proteins per 200 ml plasma and duration of chiropractic care expressed in weeks. $y = 21.5 (x) + 86$, $r = 0.34$, n = 47, p < 0.02.

**Discussion**

Oxygen-centered radicals can directly damage the structure of cellular macromolecules destined to carry out the biological functions of life, such as DNA, RNA, and protein. Thiols (sulfhydryl) content in biological tissues can react with the oxygen-centered radicals to form low molecular weight disulfides. Thiols are, therefore, primary antioxidants.

Because oxidative stress induced free radicals is a cascading chemical event, then the thiol content of plasma proteins would no doubt reflect the overall reduction/oxidation (redox) cellular balance existing in vivo at the particular time of blood sampling. Therefore, the thiol data presented in Table 1 and Fig. 1 clearly demonstrate that musculoskeletal discomfort produces a shift in the redox physiologic balance of free radical production toward an increased oxidation, which in turn is a condition well-known to induce health consequences thereof. Long-term chiropractic care of more than 2 years was shown to re-establish a normal redox physiologic state independent of sex, age, or nutritional supplements. These data were taken as evidence that as an intervention, chiropractic could influence the basic physiologic process of endogenous generation of oxidative stress, and as such may prove to be a scientific explanation behind positive health benefits being associated to chiropractic care.

If indeed chiropractic care could reverse in vivo oxidative stress, then what clinical parameters would chiropractic care be expected to influence? Most scientists now agree that the life-threatening hazards of oxidative stress are most strongly associated to aging phenomena. Hence, according to this line of reasoning chiropractic care would be expected to limit free-radical damage to DNA, RNA and proteins preventing or limiting: (i) mutation, (ii) altered genetic expression and (iii) the incidence of age-associated disorders such as immune suppression, inflammation and cancer. In this regard, the scientific logic already established is as follows: Thiols are antioxidants reducing DNA damage, stimulating DNA repair and immune function and thus they can retard at least some of the consequences of aging such as poor nutrition and autoimmune diseases.
The scientific evidence connecting thiol status to DNA repair capacity has been reviewed in detail earlier. It is based on the knowledge that the DNA repair enzyme, PARP, has a thiol containing zinc finger, and so when oxidized to disulfides it cannot bind zinc that subsequently permits binding to DNA and participation in DNA repair. As a result, increased PARP activity strongly correlates to lifespan and aging, which in turn justifies thiols as a molecular target for anti-aging therapies. The data on chiropractic care reported in this study supports that in vivo serum thiol status is a good surrogate estimate of DNA repair, and as such should be considered as another parameter of the emerging anti-aging awareness in both the public and health care communities. In fact this hypothesis is strongly supported by the fact that active diseased patients generally had plasma thiol values of less than 90 nM cysteine compared to subjects who received regular long term chiropractic care, which had corresponding values of 146 compared to non-chiropractic treated controls who had values of 124.

Conclusion
Some final observations should be noted. The results clearly support the recommendations being made for wellness care by chiropractors. In addition, it should be stated that these results occurred under normal practice conditions and indicate what most chiropractors are likely achieving when performing long term care. Also, it refutes earlier views that five to seven years of care was necessary to optimize human health status, as indicated by serum thiol levels. Our sampling indicates that this can occur in a time frame approximately half that period. Finally, there is no doubt that chiropractic care was the dominant factor in being able to realize thiol values that in some cases have not been seen in nutraceutical testing, the only other intervention shown to improve thiol values.

Our lab now plans to further confirm these effects of chiropractic care in prospective studies.

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