Sensitivity to Pain in Children With Acute Lymphoblastic Leukemia (ALL)

Manijeh Firoozi[1], Reza Rostami[1]

Abstract

Background: There are many physiological and psychological factors, which affect sensitivity to pain in children afflicted with ALL. The main purpose of this study was to evaluate the relation between salivary cortisol and sensitivity to pain, and also study the role of age and gender.

Methods: Seventy eight children (33 girls and 45 boys, aged 3 to 12 years) with ALL participated in this study. Morning salivary cortisol was measured and Behavior Scales of Sensitivity to Pain for Children (BSSPC) and Pre-Linguistic Behavioral Pain Reactivity Scale (PL-BPRS) were applied.

Results: The results showed a high significant correlation between cortisol levels and pain sensitivity. Cortisol suppression was observed in some participants. The roles of gender and age in relation between cortisol levels and sensitivity to pain were assessed by using moderated regression. Gender and age moderated the relation between sensitivity to pain and cortisol level.

Conclusion: Conditional fear can explain for high sensitivity to pain amongst the participants; chemotherapy drugs might play a role in cortisol suppression and parenthood style perhaps determines sex difference in reaction to pain.

Keywords: Child; Leukemia; Cortisol; Pain

Please cite this article as: Firoozi M, Rostami R. Sensitivity to Pain in Children With Acute Lymphoblastic Leukemia (ALL). Iran J Cancer Prev. 2012; 5(2): 74-80.

1. Dept. of Psychology, Tehran University, Tehran, Iran

Corresponding Author:
Manijeh Firoozi, PhD;
Assistance Professor of Health Psychology
Tel: (+98)21 22 95 96 35
Email: manijeh_firoozi@hotmail.com
Received: 18 Nov. 2011
Accepted: 15 Jan. 2012
Iran J Cancer Prev 2012; 2:74-80

Introduction

Children with cancer often suffer from unpredictable and uncontrollable pain. This usually drives the child and his/her family to a desperation, despondency, and distress [1]. A wide variety of diagnostic methods as well as painful and aggressive treatments are administered to children with Acute Lymphoblastic Leukemia (ALL). Above all, the most painful treatments such as injections and especially bone marrow transplantation provoke anxiety and avoidance reactions in pediatric oncology cases [2]. A painful medical process induces a wide variety of reactions in children, from avoidance reactions to a loud cry due to pain [3]. The first experience of pain may create a vicious cycle for subsequent painful experiences. The first experience of pain is along with an anxious state and anticipation of terrible experience in the similar situation [4]. Laboratory studies have also confirmed that there are obvious individual differences in reaction to pain [5]. The important point is such individual differences tend to remain constant over the time [6].

There is often a significant relationship between reaction to painful stimuli and child's temperament. For instance, difficult children have stronger reaction to pain [7]. Studies have revealed that a marked increase of cortisol levels has been observed in children undergoing aggressive treatments [8]. The cortisol level is a key concept in evaluation of sensitivity to pain. Accordingly, in this study alongside the exploring of the individual differences (age and gender) we have also scrutinized the key role of the cortisol level.

Selye proposed that level of sympathetic system activity is an opposite yardstick for the study of emotional state of individuals [9]. One of the ways to study the level of sympathetic activity is to measure the cortisol level. For instance, when anxiety is heightened, the cortisol level also increases correspondingly [10].

A number of studies have assessed the blood cortisol level changes from a developmental perspective. The activity of the Hypothalamic-Pituitary-Adrenal (HPA) axis is very intense in

children; however, it develops a downward trend until puberty. Researchers believe that the intensity in children's sympathetic nervous system activity is due to this fact that children at the start respond equally to both new stimuli and threatening stimuli; however, over the time, they learn to differentiate the differences between such stimuli and therefore to respond properly [11].

According to the relevant studies, those children who were more exposed to environmental changes demonstrated a higher activity of the HPA axis. A number of studies have indicated that those children who from the beginning have under activity or over activity of the HPA axis and the level of such activities remain either constant or gradually increase over the time are considered to be emotionally negative and socially isolated [12].

The activity of the HPA axis is also affected by cancer and its treatment. Radiation therapy is the common cause of irreversible thalamic dysfunctions in cancer patients while there has been no evidence to show that chemotherapy has lasting effects on the HPA axis [13].

Some studies have confirmed that there is a significant difference in reaction to pain between the two genders. For example, Arbuckle [14] by using the behavioral checklist observed that girls have stronger reaction to pain in compare to boys. The role of age in sensitivity to pain is a controversial issue, some researchers like Dao and Le Resche [15] have shown that sensitivity to pain decreases with increasing age; however, other researches [16] have revealed that age plays no role in sensitivity to pain. However, children at a very early age make a poorer adjustment to pain [16].

Materials and Methods

Participants

Seventy-eight children (33 girls, 45 boys) with Acute Lymphoblastic Leukemia (ALL) participated in this study. Potential participants were selected randomly from the list of Outpatient Chemotherapy Room of Mahak Children's Hospital and Hematology-Oncology Research Center. Eligibility requirement for entry into the study were: (1) children between the ages of 3 and 12 years (2) diagnosed with Acute Lymphoblastic Leukemia (ALL) (3) underwent chemotherapy, and (4) having identical protocol treatment. The patients, who went to the hospital early in the morning in order to receive chemotherapeutic drugs, were enrolled in this section. All patients received standard induction chemotherapy according to the NOPHO-92-ALL protocol.

Table 1. PLBPRS for rating the behavioral pain reactivity in children

Type 1	**Paradoxical pain reactivity** Children with cancer rarely show apparent pleasure reaction to a painful stimulus (such as smiling or laughing). This reaction reflects denying pain.
Type 2	**Absence of pain reactivity** Absence of any reactions described in class 5 with absence of nociceptive reflexes (such as absence of hand-withdrawal reflex or absence of arm-withdrawal reflex from the needle during taking chemotherapy).
Type 3	**Hypo reactivity to pain** Children with cancer sometimes show endurance of pain because of increasing psychological resiliency during their illness. After noxious stimuli, the following possible abnormalities are observed: incomplete pain reactivity compared to class 4, abnormally delayed reaction time.
Type 4	**Normal pain reactivity** After painful stimuli the following reactions are observed: cries, screams, moaning, grimaces, reflexes of nociceptive withdrawal, lack of movement, body orientation and glance towards the painful area (e.g. glance towards the venipuncture or sometimes away from it).
Type 5	**Hyper reactivity to pain** Disproportionate cries and screams given the painful stimulus (hypersensitive).

Prednisolone was administrated 60mg /m²/day for 5 weeks, tapering over 9 days. All parents along with their children were in the chemotherapy room and tried to relieve their pain. In the other words, they played role of an external emotion regulator.

Measures

Behavior Scales of Sensitivity to Pain for Children (BSSPC)

The BSSPC incorporate a rating of the intensity, frequency, and duration of each behavior as a behavior checklist. The scales assess the degree of distress caused by injection pain as well as the intensity of reaction to injection pain felt by children. This test can be carried out by a physician assistant, parents, or a researcher. Regarding the observed behavior, this could be scored from 1 to 10 on this scale. Score 1 indicates that the child is in a totally relaxed state while score 10 indicates a completely distressed state or incapacitated as the patient is too enervated to show any endurance. Some examples of rating items of this scale: children react by nonverbal expression of anxiety such as frowning and turning their face away or cry loudly and act aggressively toward the medical staff.

In order to assess the reliability of this test, the relation of the behavior scale for sensitivity to pain was examined using a Visual Analogue Scale (VAS) and a behavioral checklist, which both measure the sensitivity to pain. The Corenbach's α for each test was 0.84 and 0.89 respectively, indicating that both are desirable values. For assessing the validity of this test, the reaction to pain was measured and analyzed in 25 participants who came to the hospital twice in a week to receive the chemotherapy drugs intravenously. After twice running the test, the correlation coefficient was acceptable (r=0.78; p=0.001).

Pre-Linguistic Behavioral Pain Reactivity Scale (PL-BPRS)

The PLBPRS assesses behavioral pain reactivity by rating the patients' apparent and observable reactions to noxious stimuli. This scale is qualitative, categorizing behavioral pain reactivity in 5 classes summarized in Table 1. The PL-BPRS has been previously found to be reliable and valid for assessment of pain reactivity in children [17]. This scale takes point from 1 to 5.

Cortisol

The measurement of cortisol in saliva reliably reflects physiologically active free cortisol levels in blood, since unbound plasma cortisol diffuses easily from blood to saliva. Salivary samples were collected using the Salivette system from Sarstedt, and then centrifuged. Cortisol kits were refrigerated until retrieved by a research assistant and measured by radioimmunoassay employing commercial reagents from Diagnostic Systems Laboratories, inc. Samples were stored at 50C until using EIA kits from Salimetrics, Inc. The intra-assay coefficients of variation were 3.1 and 0.7% for low and high controls, and inter-assay values were 3.04 and 1.6%, respectively. Mean (range) morning salivary cortisol in a control group of healthy children was 11.5 (1.3-27.7) nmol/l. The value of 12 nmol/l for the lower limit of a normal stimulated cortisol in saliva was chosen as all patients tested at baseline had a normal stimulated s-cortisol > 500 nmol/l and a stimulated cortisol in saliva > 12 mol/l.

Results

Participants in this study were between 3 and 12 years old (M=7.29; SD=2.89); 57.7% of them were boys. Average salivary cortisol level was (M=16.7; SD=13.1). In addition, based on the cortisol levels, the participants were divided into three levels of cortisol, namely high, low, and normal found on experimental standard. Table 1 shows frequency and percentage of participants based on their gender and cortisol category. Table 3 shows a high correlation between cortisol level (CL) and both scales of sensitivity to pain (BSSPC×cl=0.89; p<0.001 and PL-BPRS×cl=0.93; p<0.001).

Adjusted regression analysis was used to find out whether gender and age can modify the relationship between cortisol level and sensitivity to pain.

According to Table 4, when the dependent variable is sensitive to pain, model 1 indicates gender and then age predicts cortisol level (gender: F=515.369; p<0.001 and age: F=104.264; p<0.001). Model 2 indicates gender and cortisol level as predictor variable and could modify sensitivity to pain (F=726.029; p<0.001). In addition, when age and cortisol level are predictor variables, they could modify sensitivity to pain (F=67.676; p<0.001).

The results of regression analysis of model 1 in Table 5 indicate that the relationship between gender and cortisol level with sensitivity to pain is 0.539, which explains 27 percent of the variance of reaction to pain. Model 2 shows the relationship among predictor variables (cortisol level and

gender) and sensitivity to pain after application of gender as a moderator variable. Comparison between models 1 and 2 reveals that gender increases R2 to 62.7% which is significant ($\Delta F=565.95$; $p<0.001$).

In Model 1 the results of regression analysis of age variable indicate that the relationship between age and cortisol level with sensitivity to pain is 0.858 which explains 72 percent of the variance of sensitivity to pain. Model 2 shows the relationship among predictor variables (cortisol level and age) and sensitivity to pain after adding age as a moderator variable. Comparison between models 1 and 2 demonstrate that age increases R2 to 58% which is significant ($\Delta F=491.074$; at $p=0.002$).

Table 6 shows the standard and nonstandard regression analysis coefficients based on models 1 and 2.

Regression coefficients in model 1 demonstrate that the predictor variables of cortisol level (β = 0.501, t = 4.976) and gender (β = 0.106, t = 1.056) can significantly explain the variance of sensitivity to pain. Also, regression coefficients in model 2 demonstrate that cortisol level (β = 0.153, t = 4.072), gender (β = 1.030, t = 23.790), and the adjusted effect of gender (β = 1.030, t = 23.790) significantly explain the variance of sensitivity to pain. In other words, when the level of cortisol increases, sensitivity to pain increases too; besides, being a girl explains more about sensitivity to pain.

In this study, code 1 was assigned to the variable girl and code 2 to the variable boy; negative score in gender demonstrates that as the scores move toward 1 which is a being a girl their moderating

Table 2. The number and percentage of participant based on gender and cortisol levels

GENDER	CORTISOL						TOTAL	
	Low		Normal		High			
	F	%	F	%	F	%	F	%
Boys	8	10.25	17	21.79	8	10.25	33	42.31
Girls	11	14.1	31	39.74	3	3.84	45	57.69
Grand Total	19	24.35	48	61.53	11	14.10	78	100

Table 3. The Pearson correlation between cortisol level and sensitivity to pain

Variables	BSSPC	PL-BPRS	Cortisol Level
BSSPC	1		
PL-BPRS	0.79 **	1	
Cortisol Level (CL)	0.89 **	0.93 **	1
* p <0.05	p<0.001**		

Table 4. ANOVA for determination of the significance level of models 1 and 2

	Model 1					Model 2				
	Regression		Residual			Regression		Residual		
	SS	M	SS	M	F	SS	M	SS	M	F
Gender	72.005	36.002	175.790	2.344	515.369**	227.468	65.482	20.327	60.771	726.029**
Age	182.247	91.124	65.548	0.874	104.264**	182.312	65.360	65.482	247.795	67.676**
p <0.05*			p<0.001**							

Predictors : constant, cortisol level, gender/age

Predictors : constant moderating effect, cortisol level, gender/age

Dependent variable : sensitivity to pain

Table 5. Results of regression analysis based on models 1 and 2

	Model 1					Model 2				
	R	R^2	ΔR^2	ΔF	P	R	R^2	ΔR^2	ΔF	P
Gender	0.539	0.272	0.291	15.360	0.001	0.958	0.915	0.627	565.957	0.001
Age	0.858	0.728	0.728	12.471	0.001	0.858	0.725	0.581	491.074	0.002
p <0.05*				p<0.001**						

effect increases too. Furthermore, according to Table 7 regression coefficients in model 1 demonstrate that the predicator variables of cortisol level (β = 0.504, t = 8.986) and age (β = 0.675, t = 11.364) can significantly explain the variance of sensitivity to pain. Regression coefficients in model 2 demonstrate that the cortisol level (β = 0.508, t = 8.263) and age (β = - 0.680, t = - 10.974) have become significant in relation to sensitivity to pain at the level of 0.001; also, the adjusting effect of age (β = 1.030, t = 23.790) shows significant in relation to sensitivity to pain.

Conclusion

This study demonstrated that pain sensitivity is associated with cortisol level. According to the findings of this study, 14.1% of the participants experienced an elevated level of cortisol. These findings also confirm the results of other studies about high-level injection phobia in children with cancer [18]. A conditioned fear causes to keep the cortisol level high. When children are faced to a new (unfamiliar) or threatening situation, until normalization will take place, cortisol is increasingly secreted [19]. Chemotherapy room as a threatening situation is a cue for experiencing of pain. For children with genetic vulnerability, reaction to pain resumes with encounter to these kinds of cues.

According to the results, 61.5% of the participants exhibited a normal level of cortisol. The findings are in compliance with the other studies confirming that children with cancer show a good adjustment [20]. Some research studies have demonstrated that children with cancer show normal emotional responses and even outperform their healthy peers. Phipps [21] Dejong and Vannatta, Gartste and Noll [22], and Bennett [23] showed that children with cancer displayed milder anxiety symptoms and depression. Normal cortisol level confirms these findings. There are also other theories that contribute to the explanation of this phenomenon. For example, children may have devolved a learned helplessness because of the inevitability of painful treatments. Such helplessness makes children succumb instead of confrontation or escape, which in turn keeps the cortisol level low. Selye [9] pointed out that sympathetic system activity is provoked by the necessity to produce fight-or-flight response. If fight-or-flight response is evaluated inoperative, cortisol level will not rise.

A small number of participants (24.4% which is meaningful) exhibited a low-level cortisol. Chemotherapy drugs are responsible for cortisol suppression [24] and cortisol suppression is responsible for low level of vitality in children with cancer in compare to peers [25]. A decrease in salivary or blood cortisol rate leads to negative memory impairment. The children with Acute Lymphoblastic Leukemia recalled less negative than positive pictures in compare to healthy children [26]. However, carefully controlled experiments are needed to detect cause(s) of cortisol suppression.

The results of this study suggest that gender plays a moderating role in the relation between cortisol

Table 6. Standard and nonstandard regression analysis coefficients based on models 1 and 2 (gender)

Model		B	SE	β	t	P
1	Constant	3.755	0.743		0.052	0.001
	Cortisol level	0.096	0.019	0.501	4.976	0.294
	Gender	- 0.384	0.363	- 0.106	- 1.056	0.001
2	Constant	4.366	0.256		17.074	0.001
	Cortisol level	0.029	0.007	0.153	4.072	0.001
	Gender	-2.751	0.159	- 0.763	- 17.267	0.001
	adjusting effect of gender	0.583	0.024	1.030	23.790	0. 001

Table 7. Standard and nonstandard regression analysis coefficients based on models 1 and 2 (age)

Model		B	SE	β	t	P
1	Constant	4.718	0.106		44.571	0.001
	Cortisol level	0.905	0.107	0.504	8.985	0.001
	Age	-1.211	0.111	-0.6756	-11.364	0.001
2	Constant	4.719	0.107		44.262	0.001
	Cortisol level	0.912	0.110	0.508	8.263	0.001
	Age	-1.219	0.111	-0.680	-10.974	0.001
	Adjusting effect of age	0.036	0.132	0.017	6.272	0.05

level and sensitivity to pain. Girls in compare to boys showed a higher cortisol level and displayed more sensitivity to pain. These findings corroborate the results of other relevant researches [27]. A wide variety of factors can contribute to this difference for instance, the irritability of nerve cells increases the number of nerve receptors in skin [28, 29].

The second goal of this study was to conduct an analysis to assess whether age and gender can moderate the relation between pain sensitivity and cortisol level. Based on the findings of this study, younger children and girls showed a higher sensitivity to pain. It seems these participants have more biological preparedness for more severe reaction to pain. Evidence from recent epidemiologic studies clearly demonstrates that girls are at substantially greater risk for many clinical pain conditions, and there is some suggestion that postoperative and procedural pain may be more severe among girls than boys [30, 31]. Girls rated their chronic pain as more intense on a VAS than boys [32].

According to the findings of this study, there is a discrepancy between the cortisol level and sensitivity to pain of boys and girls. Parents exert different parenthood styles to boys and girls. They give extra attention to reactions to pain made by girls and also they reinforce such reactions and their continuation. It seems that emotion regulation styles are different in boys and girls. Girls conceivably eliminate the negative effect of such emotions through externalization of negative emotions while boys minimize negative emotions through trivializing their pain. The findings of this study indicate that age is important in sensitivity to pain as well.

Tordjman et al [33] showed that age was associated with reaction to pain negatively. Younger children reported more anxiety and heart rate as well as marginally higher procedural anxiety and observed distress. However, other studies could not find a significant relation between age and pain [34]. This gap originates from the range of age in different studies. Children under one year old are vulnerable to distress when they experience intolerable pain due to psychological issues such as serious attachment impairment.

Some considerable evidences emphasize that sensitivity to pain and its distress remains constant over the time [35] therefore, it is necessary to find interventions to control distress resulting from sensitivity to pain. One of the limitations of this study was a relatively small number of participants, so it is recommended that in addition to study a larger statistical population, the roles of other factors influencing sensitivity to pain in children with cancer should be evaluated.

Acknowledgment

We would like to thank the children with cancer from Mahak Hospital who participated in this study.

Conflict of Interest

Nothing to declare.

Authors' Contribution

Manijeh Firoozi and Reza Rostami designed the study, collected the data, analyzed the data and wrote the paper.

References

1. Gerali M, Servitzoglou M, Paikopoulou D, Theodosopoulou H, Madianos MM, Vasilatou-Kosmidis H. Psychological Problems in Children With Cancer in the Initial Period of Treatment. Cancer Nursing. 2011; 34 (4): 269-76.

2. Blount RL, Piira T, Cohen LL. Management of pediatric pain and distress due to medical procedures. In M. C. Roberts (Ed.), Handbook of pediatric psychology. New York: Guilford Press. 2003; 216–33.

3. Kuppenheimer WG, Brown RT. Painful procedures in pediatric cancer: A component of interventions. Clinical Psychological Review. 2002; 22: 753-86.

4. Liossi C, Hatira P. Clinical hypnosis in the alleviation of procedure-related pain in pediatric oncology patients. International Journal of Clinical and Experimental Hypnosis. 2003; 51(1): 4–28.

5. Broome ME, Bates TA, Lillis PP, McGahee TW. Children's medical fears, coping behaviors, and pain perceptions during a lumbar puncture. Oncology Nursing Forum. 2005; 17(3): 361–7.

6. Cavender K, Goff MD, Hollon E, Guzzetta CE. Personal deferent in children during venipuncture: Effects on children's pain, fear, and distress. Journal of Holistic Nursing. 2004; 22(1): 32-56.

7. Ranger M, Campbell-Yeo M. Temperament and Pain Response: A Review of the Literature. Pain management nursing. 2008; 9(1): 2-9.

8. Herrington CJ, Olomu IN, Geller SM. Salivary Cortisol As indicators of Pain in Preterm Infants A Pilot Study, Clinical nursing research. 2005; 13(1): 53-68.

9. Selye H. Stress and the general adaptation syndrome. British medicine journal. 1950; 1(4667): 1385-92.

10. Ahs F, Furmark T, MichelGard A, Langstrom B, Apple L, Wolf OT, et al. Hypothalamic Blood Flow Correlates Positively With Stress-Induced Cortisol Levels in Subjects With Social Anxiety Disorder. Psychosomatic Medicine. 2006; 68(6): 859–62.

11. Gunnar MR, Brodersen L, Krueger K, Rigatuso J. Dampening of adrenocortical responses during infancy:

Normative changes and individual differences. Child Development. 2006; 67(3): 877–89.

12. Gunnar M, Quevedo K. The neurobiology of stress and development. Annual Review of Psychology. 2007; 58: 145–73.

13. Lointier P, Wildrick DM, Boman BM. The effects of hormones on a human colon cancer cell line in vitro. Anticancer Research. 1992; 12(4): 1327–30.

14. Lynch AM, Zuck SK, Goldschneider KR, Jones BA. Sex and Age Differences in Coping Styles Among Children with Chronic Pain. Journal of pain and symptom management. 2007; 33(2): 208-16.

15. Knutsson U, Dahlgren J, Marcus C, Rosberg S, Bronnegard M, Stierna P, et al. Circadian Cortisol Rhythms in Healthy Boys and Girls: Relationship with Age, Growth, Body Composition, and Pubertal Development. The Journal of Clinical Endocrinology & Metabolism. 1997; 82(2): 536-40.

16. Dao TT, LeResche L. Gender and age differences in pain. J Orofac Pain. 2002; 14:169–84.

17. Tordjman S, Antoine C, Cohen DJ, Gauvain-Piquard A, Carlier M. Study of the relationships between self-injurious behavior and pain reactivity in infants. Encephale. 1999; 25(2): 122-34.

18. Veenema AH, Meijer OC, Kloet ER, Koolhaas JM. Genetic selection for coping style predicts stressor susceptibility. Journal of Neuroendocrinology. 2003; 15(3): 256–67.

19. Lam S, Dickerson SS, Zoccola PM, Zaldivar F. Emotion regulation and cortisol reactivity to a social-evaluative speech task. Psychoneuroendocrinology. 2009; 34(9): 1355-62.

20. Phipps S, Larson S, Long A, Rai SN. Adaptive style and symptoms of posttraumatic stress in children with cancer and their parents. Journal of Pediatric Psychology. 2006; 31(3): 298–309.

21. Dejong M, Fombonne E. Depression in pediatric cancer: An overview. Psycho-Oncology. 2006; 15(7): 553–66.

22. Vannatta K, Gartstein MA, Short A, Noll RB. A controlled study of peer relationships of children surviving brain tumors: Teacher, peer, and self ratings. Journal of Pediatric Psychology. 1998; 23(5): 279–87.

23. Bennett DS. Depression among children with chronic medical problems: A meta-analysis. Journal of Pediatric Psychology. 1994; 19(2): 149–69.

24. Ng AC, Kumar SK, Russell SJ, Rajkumar SV, Drake MT. Dexamethasone and the risk for adrenal suppression in multiple myeloma. Lukemia. 2009; 23 (5): 1009-11.

25. Firoozi M, Besharat AM, Farahani H, Ghaead Rahmat A. "Vitality", a missing link, in adjustment to childhood cancer. Iranian Journal of cancer prev. 2011; 4(3): 109-13.

26. Firoozi M, Besharat AM, Pour Naghash Tehrani S. Cognitive emotion regulation in children with acute lymphoblastic leukemia (ALL). Iranian Journal of cancer prevention. 2011: 4(4): 159-61.

27. Arbuckle TE. Are there sex and gender differences in acute exposure to chemicals in the same setting? Environmental research. 2006; 101(2): 195-204.

28. Gilkey DP, Keefe TJ, Peel JL, Kassab OM, Kennedy CA. Risk Factors Associated With Back Pain: A Cross-Sectional Study of 963 College Students. Journal of Manipulative and Physiological Therapeutics. 2010; 33(2): 88-95.

29. Costigan M, Scholz J, Woolf CJ. Neuropathic Pain: A Maladaptive Response of the Nervous System to Damage. Annual Review Neuroscience. 2009; 32:1–32.

30. Zacny JP, Beckman NJ. The effects of a cold-water stimulus on butorphanol effects in males and females. Pharmacol Biochem Behav. 2004; 78(4): 653–9.

31. Wise EA, Price DD, Myers CD, Heft MW, Robinson ME. Gender role expectations of pain: Relationship to experimental pain perception. Pain. 2002; 96(3): 335–42.

32. Wiesenfeld-Hallin Z. Sex differences in pain perception. Gend Med. 2005; 2(3): 137–45.

33. Dao TT, LeResche L. Gender and age differences in pain. J Orofac Pain. 2002; 14(3): 169–84.

34. Aslaksen PM, Myrbakk IN, Hoifodt RS, Flaten MA. The effect of experimenter age and gender on autonomic and subjective responses to pain stimuli. Pain. 2007; 129(3): 260–8.

35. Vetrhus M, Berhane T, Soreide O, Sondenaa K. Pain persists in many patients five years after removal of the gallbladder: Observations from two randomized controlled trials of symptomatic, noncomplicated gallstone disease and acute cholecystitis. J Gastrointest Surg. 2005; 9(6): 826–31.