British Journal of Medicine & Medical Research
19(5): 1-8, 2017; Article no.BJMMR.30663
ISSN: 2231-0614, NLM ID: 101570965

SCIENCEDOMAIN *international*
www.sciencedomain.org

Salivary Biomarkers to Monitor Osteopenia and Stress Levels in Breast Cancer Survivors

Xun Xiao[1], Michael C. W. Yip[2], Wings T. Y. Loo[2,3*], Liang Hao[1], Louis W. C. Chow[3], Erich F. Lie[3], Christopher Y. C. Chow[3] and Min Wang[1*]

[1]The State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University, Sichuan, People's Republic of China.
[2]Department of Psychological Studies, The Education University of Hong Kong, Hong Kong.
[3]UNIMED Medical Institute and Organisation for Oncology and Translational Research, Hong Kong.

Authors' contributions

This work was carried out in collaboration between all authors. Authors XX, MCWY, WTYL and MW designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors LWCC and CYCC managed the analyses of the study. Authors LH and EFL wrote the final draft of manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2017/30663
Editor(s):
(1) Evangelos Marinos, University of Athens, School of Medicine, Laboratory of Biology, Athens, Greece.
Reviewers:
(1) Bruno Lopes Cançado Machado, Pro Uro Urological Center, Brazil.
(2) Natt Makul, Phranakhon Rajabhat University, Bangkok, Thailand.
(3) Monika Zajkowska, Medical University, Bialystok, Poland.
(4) Yu Koyama, Niigata University, Graduate School of Health Sciences, Japan.
Complete Peer review History: http://www.sciencedomain.org/review-history/17399

Original Research Article

Received 24th November 2016
Accepted 27th December 2016
Published 31st December 2016

ABSTRACT

Aims: The aim of this study is to assess salivary biomarkers, i.e. cortisol, calcium, phosphate, osteocalcin, vitamin D and estradiol levels, to monitor osteopenia and stress levels in post-treatment breast cancer patients.
Methods: The salivary biomarkers of forty-five female breast cancer survivors aged between 30 to 48 years were compared against twenty-eight disease-free, healthy female subjects, which act as the reference values in our study. Saliva collection was done by resting/drooling collection method (minimal oral movements). The independent unpaired t-test was used to compare the differences between the parameters of control group and patient group.
Results: The salivary flow rate and the amount of saliva were not significantly different between both groups. The concentration of salivary cortisol in breast cancer survivors was significantly

**Corresponding author: E-mail: wtyloo@gmail.com, 1283132362@qq.com;*

higher compared to healthy controls (P<0.01). The mean concentrations of salivary calcium (P<0.01), phosphate (P<0.05), osteocalcin (P<0.001), vitamin D (P<0.001) and estradiol (P<0.05) in the breast cancer survivor group were significantly lower than those in the control group. **Conclusion:** Our findings suggest that the measurement of salivary biomarkers can be considered as a useful method to monitor osteopenia and stress levels in breast cancer survivors.

Keywords: Breast cancer survivor; osteopenia; stress; saliva; biomarkers.

1. INTRODUCTION

Progress in screening, detection and therapy for breast cancer have resulted in increased survival rates [1,2], especially in North America and several European countries over the past 30 years [3,4]. Increasing number of women are long-term survivors of breast cancer with an array of quality-of-life issues that affect their daily living in both short- and long-term survivorship.

Bone health is very important in breast cancer patients because certain medications used to treat breast cancer can cause bone loss, in particular hormonal therapy (such as aromatase inhibitors) and chemotherapy. Additionally, previous studies have demonstrated that breast cancer patients treated with adjuvant chemotherapy are more likely to undergo a premature menopause accompanied by an acceleration of bone loss [5,6]. As a consequence, a higher risk for osteoporosis exists in women who have had breast cancer [7]. As bone density decreases, the risk of fracture increases. This can include fractures of the wrist, femur, and vertebrae. Thus, it is necessary to monitor the impact of cancer treatment on bone health and the morbidity associated with cancer treatment-induced bone loss (CTIBL).

Breast cancer survivors also experience a high level of depressive and anxious symptoms [8]. The severity of symptoms associated with the difficulty to cope with the diagnosis and treatment of cancer vary between mild to severe depression; some women with breast cancer even fulfilled the criteria of clinical depression at 18 months post-diagnosis [9]. Although these symptoms are frequently experienced from the point of diagnosis, most women diagnosed with breast cancer are still at risk after a period of recovery [10,11]. Intriguingly, chronic stress may be associated with the pathogenesis of the cancer itself; it might have an impact to cancer growth and metastasis [12].

Various psychosocial studies have been conducted to assess the stress levels in breast cancer survivors, however these often relied on questionnaires [13-15]. Plasma cortisol may be used to monitor stress levels [16,17]; however, blood sampling may have the risk of infection, complications when the precautions were not well carried. Recently, the use of salivary biomarkers has been validated from oral diseases to systemic diseases [18-20], broadening the potential for systemic disease detection [21]. Saliva-based translational research and technology is now at its mature juncture and it should be further evaluated to determine its utility for breast cancer management. The aim of this study is to assess salivary biomarkers, i.e. cortisol, calcium, phosphate, osteocalcin, vitamin D and estradiol levels, to monitor osteopenia and stress levels in post-treatment breast cancer patients.

2. MATERIALS AND METHODS

2.1 Subject Information and Study Design

Forty-five females aged between 30 to 48 years who underwent a modified mastectomy for invasive ductal carcinoma and were treated with 4 cycles of chemotherapy, were recruited from UNIMED Medical Institute, Hong Kong. The control group consisted of twenty-eight disease-free, healthy female subjects, aged 26 to 38 years, recruited from the community. As reliable reference values for salivary biomarkers are yet to be determined, the values obtained from the healthy subjects act as the reference point in our study. All subjects gave consent prior to enrollment, and the study protocol was approved by the ethics committee of the University of Hong Kong. Exclusion criteria of control patients included a diagnosis of any malignancies within 5 years prior to saliva collection.

2.2 Saliva Collection

To avoid food contamination on saliva biomarkers level, saliva samples were collected between 10 am to 12 am before lunch. Unstimulated saliva samples were consistently collected, stabilized, and preserved as described

by Zhang [22] (Fig. 1). The participants were allowed to rinse their mouth with water prior to collection, and wait for 10 minutes before commencing saliva collection. In this study, resting/drooling collection method (minimal oral movements) was used to collect whole mouth saliva from the oral cavity. All subjects were asked to sit comfortably in an upright position and tilt their heads slightly down to pool saliva in the mouth. A sterilized cotton ball was placed under the tongue for at least 10 minutes until the cotton ball is fully soaked with saliva. The cotton ball was then transferred into a syringe which was assembled with a micro-tube. 3 mL of saliva was collected into pre-labelled micro-tube by re-attaching the plunger rod to the syringe and pushing the plunger rod. The sampling process was monitored by the nursing staff. All saliva samples were immediately centrifuged at 10000 g for 10 min to eliminate food, debris, and unwanted substance from contaminating the sample that may cause analytical inaccuracy. The supernatant was transferred into a fresh Eppendorf tube and appropriately labelled. After pre-processing, all samples were stored at −80°C until analysis.

2.3 Measurement of Salivary Cortisol, Calcium, Phosphate, Osteocalcin, Vitamin D and Estradiol

Cortisol values were quantified by in duplicate with enzyme-linked immunoassay (IBL, Germany). 50 µl of the saliva was pipetted into a 96-well plate, according to the manufacturer's protocol. Plate was then read at 450 nm wavelength using Universal Microplate Reader (Sunrise, Tecan Co., Grödingen, Austria). Calcium, phosphate, osteocalcin, vitamin D and estradiol concentrations in saliva were measured by Cobas Integra 400 Plus autoanalyzer (Roche, United States).

2.4 Statistical Analysis

For statistical analysis, the data are presented as means ± standard deviation. All calculations were performed using SPSS version 16.0. The independent unpaired t-test was applied to compare the differences between biological parameters of the control group and patient group. A *P*-value of less than 0.05 was considered statistically significant.

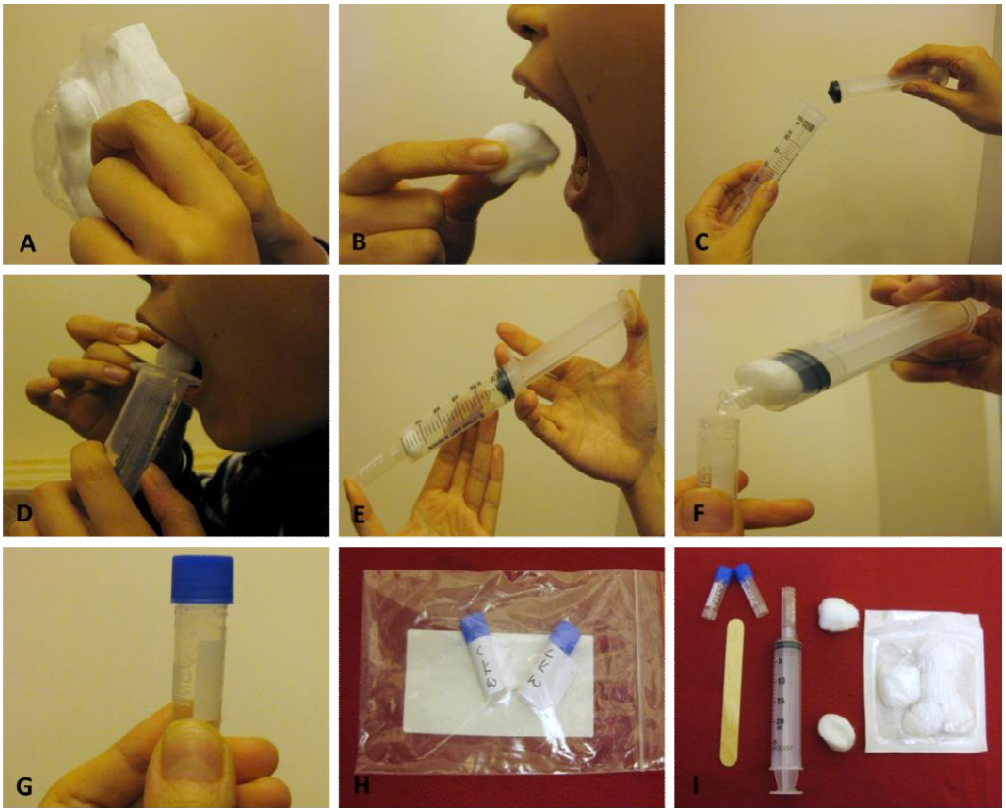

Fig. 1. Saliva collection method

3. RESULTS

3.1 Patient Characteristics

The mean age of the breast cancer survivors group is 40.2 years (age range 30-48 years). The subjects participating in this study were ethnically Chinese. All subjects in the breast cancer survivors group had premature menopause.

3.2 The Salivary Flow Rates and Amount of Saliva

The salivary flow rates in the control group and breast cancer survivor group by resting/drooling collection method was 0.39±0.10 mL/min and 0.42±0.15, respectively. There was no significant difference of salivary flow rate between both groups (P>0.05). The amount of saliva in the control group and breast cancer patient group was not significantly different (1.8±0.15 mL and 1.1±0.10, respectively, P>0.05).

3.3 Concentration Levels of Salivary Cortisol, Calcium, Phosphate, Osteocalcin, Vitamin D and Estradiol

In the breast cancer survivor group, the concentration of salivary cortisol was 23.41±6.54 nmol/L. Compared with the cortisol level of healthy subjects (10.71±3.08 nmol/L), the mean concentration of salivary cortisol in breast cancer survivors was significantly higher (P<0.01). The mean concentrations of bone biomarkers in the breast cancer survivor group and healthy subjects were described in Table 1. As seen in Fig. 2, significantly lower mean concentrations of salivary calcium (P<0.01), phosphate (P<0.05), osteocalcin (P<0.001), vitamin D (P<0.001) and estradiol (P<0.05) were found in the breast cancer survivor group.

4. DISCUSSION

The pathophysiology of CTIBL has been linked to the use of chemotherapy and aromatase inhibitors. Chemotherapy leads to an unspecific increase in bone resorption, whereas aromatase inhibitors are associated with a decrease in bone mineral density (BMD) and increased fracture risk [23]. In breast cancer survivors who experienced premature menopause or treatment-induced menopause, the lack of estrogen will further aggravate the development of osteoporosis. A significant decrease of BMD at the lumbar spine and hip was reported in Caucasian women aged 36 to 55 years, with breast cancer and artificially-induced menopause, who are treated with tamoxifen 20 mg daily [24]. Similar results were observed at femoral necks; 2 years after six cycles of cyclophosphamide, methotrexate, and fluorouracil (CMF) therapy, the decrease in the BMD of lumbar spine and femoral neck of premenopausal breast cancer patients were -5.9% and -2.0% [25]. Furthermore, aromatase inhibitor-associated bone loss (AIBL) was reportedly twice as high compared to healthy postmenopausal women [26].

In the present study, breast cancer survivors were demonstrated to have significantly lower concentration levels of salivary calcium, phosphate, osteocalcin, and vitamin D, as compared to our reference values obtained from the healthy controls. Although saliva contains analytes in concentrations that are 1000-fold less than those in blood [27], the sensitivity and specificity of using salivary calcium to identify women with osteoporosis were reported to be 67.5% and 60% respectively [28]. The results of this study demonstrated that salivary concentrations of bone markers can be used to monitor osteopenia, however, assessment should be made carefully. Previous studies have reported that conditions such as repetitive calculus formation or chronic periodontitis may affect the concentrations of salivary calcium or phosphate [29,30].

Stress is another common symptom experienced by breast cancer patients and survivors. It is frequently experienced by women with breast cancer from the point of diagnosis and persists after a period of recovery [10,11]. Previous studies have demonstrated that plasma cortisol may be used to monitor stress levels [16,17], however, it is impractical and inconvenient to regularly monitor using blood tests. In this study, the salivary cortisol concentration level in breast cancer survivors was significantly increased when compared to our reference values obtained from the healthy controls, which might be translated to higher stress levels in breast cancer survivors. The saliva/plasma ratio is previously reported to be around 1/30 [31]. Taken before lunch, the mean cortisol level of the healthy subjects taken in our study was 13 nmol/L; this is consistent with the previous studies demonstrating that the average salivary cortisol level varies between 15 nmol/L at waking up to 3 nmol/L at bed time [32]. The measurement of salivary cortisol has been argued to be better than serum cortisol, since salivary cortisol

correlates highly with free blood cortisol, whereas serum cortisol represents total cortisol instead of the free, biologically fraction [33,34]. Nevertheless, salivary cortisol measures can still be affected by varying instances that control hypothalamus—pituitary—adrenal axis reactivity and their respective modulators, receptors, or binding proteins [35], thus, the monitoring of stress levels by measuring salivary cortisol levels should consider these factors as well.

Table 1. Salivary concentrations of bone biomarkers in breast cancer survivors and healthy subjects

Biomarker	Healthy subjects (N=28)	Breast cancer survivors (N=45)
Calcium* (mmol/L)	0.86±0.44	0.46±0.25
Estradiol* (ug/mL)	0.91±0.44	0.36±0.17
Osteocalcin* (ng/mL)	4.59±0.76	1.36±0.36
Phosphate* (mmol/L)	4.45±2.16	2.49±1.17
Vitamin D* (ng/mL)	52.49±5.01	33.42±4.24

Values are presented as mean±standard deviation.
**statistically significant difference between the two groups.*
P-value of less than 0.05 was considered statistically significant

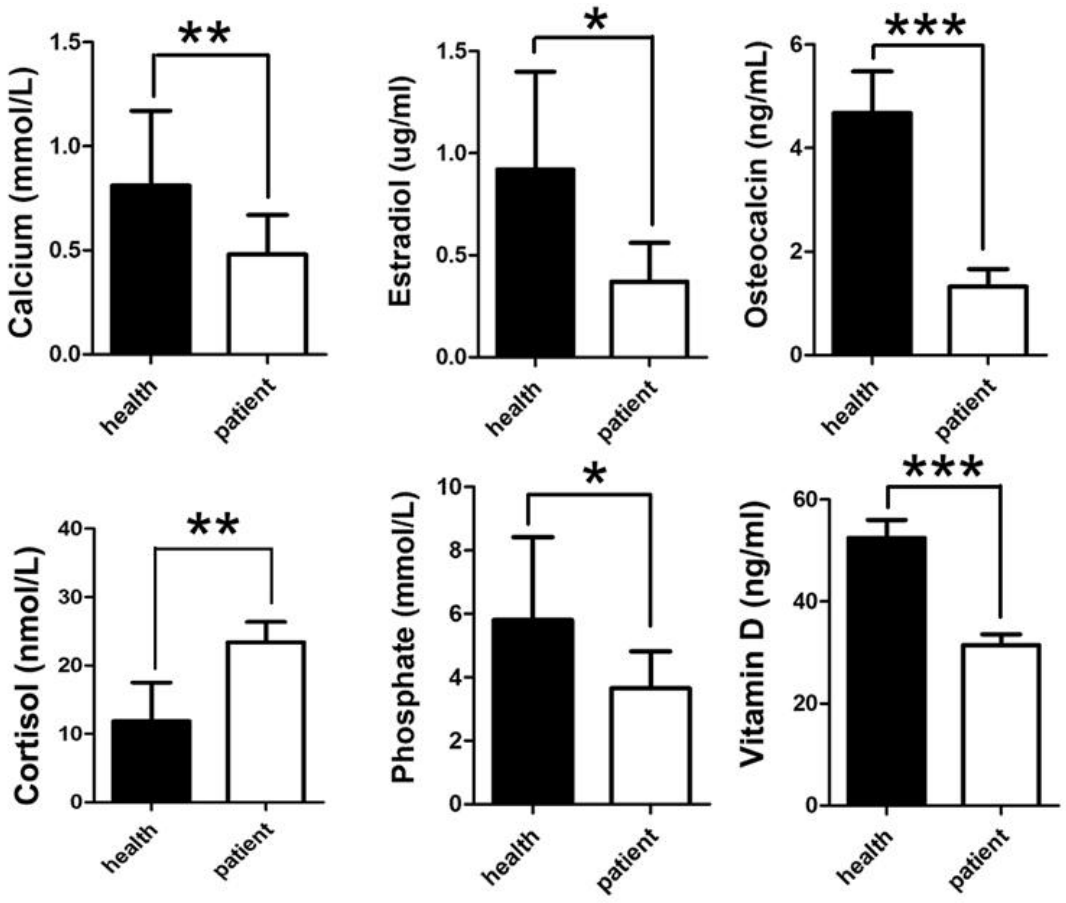

Fig. 2. The concentration levels of biomarkers measured in saliva

*Different biomarkers were detected by using samples from healthy controls (health) and breast cancer survivors (patient) groups. Salivary calcium, estradiol, osteocalcin, phosphate and vitamin D were significantly lower in the patient group as compared to control group. Salivary cortisol was significantly higher in the patient group as compared to control group. *P<0.05; **P<0.01; ***P<0.001*

Saliva has recently aroused growing interests as a diagnostic fluid since it harbors a wide spectrum of proteins/peptides, nucleic acids, electrolytes, and hormones originating from multiple local and systemic sources. Compared with blood sampling, the advantages of saliva sampling are non-invasive, low cost, and simple procedures which do not require skilled personnel. Saliva can be collected by unstimulated (resting/drooling) or stimulated conditions [36], whilst the salivary composition can be influenced by the method of collection and the degree of stimulation of salivary flow [27,37]. As cortisol levels peak at varying habitual wake time [38], we tried to standardize the time of collection in our subjects between 10 to 12 am. Additionally, we did the collection before lunch time to avoid food contamination. Unstimulated saliva stimulation method (resting/ drooling with minimal oral movements) was chosen to maintain consistency in the saliva samples.

Several limitations exist in this study. First, we had a small sample size and we were unable to obtain baseline characteristics such as T-score. Second, blood sample was not collected in this study, hence we were unable to compare salivary and serum concentrations of the biomarkers. Finally, as sampling was monitored by the nursing staff, we are certain regarding the collection compliance in this study; however, the exact timing varied due to the procedures of registration and dental exams.

5. CONCLUSION

In conclusion, our findings suggest that the measurement of salivary biomarkers can be considered as a useful method to monitor osteopenia and stress levels in breast cancer survivors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Berry DA, Cronin KA, Plevritis SK, Fryback DG, Clarke L, Zelen M, et al. Effect of screening and adjuvant therapy on mortality from breast cancer. N Engl J Med. 2005;353(17):1784-92.
2. Mayer EL, Gropper AB, Neville BA, Partridge AH, Cameron DB, Winer EP. Breast cancer survivors' perceptions of survivorship care options. J Clin Oncol. 2012;30(2):158-63.
3. Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. Cancer Epidemiol Biomarkers Prev. 2010;19(8): 1893-907.
4. Sant M, Francisci S, Capocaccia R, Verdecchia A, Allemani C, Berrino F. Time trends of breast cancer survival in Europe in relation to incidence and mortality. Int J Cancer. 2006;119(10):2417-22.
5. Cameron DA, Douglas S, Brown JE, Anderson RA. Bone mineral density loss during adjuvant chemotherapy in pre-menopausal women with early breast cancer: Is it dependent on oestrogen deficiency? Breast Cancer Res Treat. 2010;123(3):805-14.
6. Christensen C, Cronin-Fenton D, Frøslev T, Hermann AP, Ewertz M. Change in bone mineral density during adjuvant chemotherapy for early-stage breast cancer. Support Care Cancer. 2016;24(10): 4229-36.
7. Peppone LJ, Mustian KM, Rosier RN, Carroll JK, Purnell JQ, Janelsins MC, et al. Bone health issues in breast cancer survivors: A medicare current beneficiary survey (MCBS) study. Support Care Cancer. 2014;22(1):245-51.
8. Wang F, Liu J, Liu L, Wang F, Ma Z, Gao D, et al. The status and correlates of depression and anxiety among breast-cancer survivors in Eastern China: a population-based, cross-sectional case-control study. BMC Public Health. 2014;14:326.
9. Chen X, Zheng Y, Zheng W, Gu K, Chen Z, Lu W, et al. Prevalence of depression and its related factors among Chinese women with breast cancer. Acta Oncol. 2009;48(8): 1128-36.
10. Burgess C, Cornelius V, Love S, Graham J, Richards M, Ramirez A. Depression and anxiety in women with early breast cancer: five year observational cohort study. BMJ. 2005;330(7493):702.
11. Stanton AL, Bower JE. Psychological adjustment in breast cancer survivors. Adv Exp Med Biol. 2015;862:231-42.
12. Moreno-Smith M, Lutgendorf SK, Sood AK. Impact of stress on cancer metastasis. Future Oncol. 2010;6(12):1863-81.
13. Wu S, Wang R, Zhao Y, Ma X, Wu M, Yan X, et al. The relationship between self-

rated health and objective health status: a population-based study. BMC Public Health. 2013;13(320).

14. Pusic AL, Cemal Y, Albornoz C, Klassen A, Cano S, Sulimanoff I, et al. Quality of life among breast cancer patients with lymphedema: A systematic review of patient-reported outcome instruments and outcomes. Journal of Cancer Survivorship. 2013;7(1):83–92.
15. Frendl DM, Ware JE Jr. Patient-reported functional health and well-being outcomes with drug therapy: A systematic review of randomized trials using the sf-36 health survey. Med Care. 2014;52(5):439–445.
16. Hirokawa K, Taniguchi T, Fujii Y. Job stress and agentic-communal personality traits related to serum cortisol levels of male workers in a Japanese medium-sized company: A cross-sectional study. Int J Behav Med. 2015;22(1):11-7.
17. Yan YX, Dong J, Liu YQ, Zhang J, Song MS, He Y, et al. Association of suboptimal health status with psychosocial stress, plasma cortisol and mRNA expression of glucocorticoid receptor α/β in lymphocyte. Stress. 2015;18(1):29-34.
18. Hu S, Arellano M, Boontheung P, Wang J, Zhou H, Jiang J, et al. Salivary proteomics for oral cancer biomarker discovery. Clin Cancer Res. 2008;14(19):6246-52.
19. Park NJ, Zhou H, Elashoff D, Henson BS, Kastratovic DA, Abemayor E, et al. Salivary microRNA: discovery, characterization, and clinical utility for oral cancer detection. Clin Cancer Res. 2009; 15(17):5473-7.
20. Zhang L, Farrell JJ, Zhou H, Elashoff D, Akin D, Park NH, et al. Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. Gastroenterology. 2010;138(3):949-57.
21. Gao K, Zhou H, Zhang L, Lee JW, Zhou Q, Hu S, et al. Systemic disease-induced salivary biomarker profiles in mouse models of melanoma and non-small cell lung cancer. PLoS One. 2009;4(6):e5875.
22. Zhang L, Xiao H, Karlan S, Zhou H, Gross J, Elashoff D, et al. Discovery and preclinical validation of salivary transcriptomic and proteomic biomarkers for the non-invasive detection of breast cancer. PLoS One. 2010;5(12):e15573.
23. Hadji P. Cancer Treatment-Induced Bone Loss in women with breast cancer. Bonekey Rep. 2015;4:692.
24. Delmas PD, Balena R, Confravreux E, Hardouin C, Hardy P, Bremond A. Bisphosphonate risedronate prevents bone loss in women with artificial menopause due to chemotherapy of breast cancer: A double-blind, placebo-controlled study. J Clin Oncol. 1997;15(3):955-62.
25. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Coleman R, Powles T, Paterson A, Gnant M, Anderson S, et al. Adjuvant bisphosphonate treatment in early breast cancer: Meta-analyses of individual patient data from randomised trials. Lancet. 2015;386(10001):1353-61.
26. Kalder M, Hadji P. Breast cancer and osteoporosis - management of cancer treatment-induced bone loss in postmenopausal women with breast cancer. Breast Care (Basel). 2014;9(5): 312-7.
27. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. Diagnostic potential of saliva: current state and future applications. Clin Chem. 2011;57(5):675-87.
28. Rabiei M, Masooleh IS, Leyli EK, Nikoukar LR. Salivary calcium concentration as a screening tool for postmenopausal osteoporosis. Int J Rheum Dis. 2013; 16(2):198-202.
29. Moghadam SA, Zakeri Z, Fakour SR, Moghaddam AA. Does Salivary Calcium and Phosphate Concentrations Adequately Reflect Bone Mineral Density in Patients with Chronic Periodontitis? Glob J Health Sci. 2016;8(10):56723.
30. Hassan ShA, Al–Sandook TA. Salivary calcium concentration in patients with high incidence of calculus formation. Al–Rafidain Dent J. 2005;5(1): 88-90.
31. Inder WJ, Dimeski G, Russell A. Measurement of salivary cortisol in 2012–laboratory techniques and clinical indications. Clinical endocrinology. 2012; 77(5):645-51.
32. Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wong DT. Salivary biomarkers: Toward future clinical and diagnostic utilities. Clinical microbiology reviews. 2013;26(4):781-91.
33. Kosák M, Hána V, Hill M, Simunková K, Lacinová Z, Krsek M, et al. serum cortisol seems to be a more appropriate marker for adrenocortical reserve evaluation in ACTH test in comparison to salivary cortisol. Physiological Research. 2014;63(2):229.

34. Bozovic D, Racic M, Ivkovic N. Salivary cortisol levels as a biological marker of stress reaction. Med Arch. 2013;67(5):374-7.
35. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology. 2009;34(2):163-71.
36. Navazesh M. Methods for collecting saliva. Ann N Y Acad Sci. 1993;694:72-7.
37. Kaufman E, Lamster IB. The diagnostic applications of saliva-a review. Crit Rev Oral Biol Med. 2002;13(2):197-212.
38. Boivin DB, Boudreau P. Impacts of shift work on sleep and circadian rhythms. Pathol Biol (Paris). 2014;62(5):292-301.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17399