Hacettepe University Journal of the Faculty of Pharmacy
Volume 28 / Number 1 / January 2008 / pp. 1-14

Assessment of DNA Damage in Peripheral Blood Lymphocytes From Patients with Benign and Malignant Thyroid Disorders

Received : 13.03.2008
Revised : 05.08.2008
Accepted : 19.08.2008

Ülkü Ündeğer[*o], Tevfik Tolga Şahin[**], Osman Yüksel[**], Hasan Bostancı[**], Osman Kurukahvecioğlu[**], Ferit Taneri[**], Nurşen Başaran[*]

Introduction

Cancer as a disease is intimately associated with DNA damage at multiple levels[1, 2]. Elevated DNA damage levels or unrepaired DNA damage and suboptimal DNA repair may cause mutations or chromosomal aberrations that contribute to malignant transformation and cancer risk. At the cellular and molecular levels, genes whose products participate in complex responses to DNA damage are commonly deregulated or inactivated in tumours[1, 2]. Such defects in DNA damage recognition, signaling and/or downstream responses, including cell-cycle checkpoints, DNA repair or cell death, allow the genetically unstable cancer cells to survive, proliferate and acquire even more genetic instability[1-5].

Thyroid malignancies are frequently encountered tumours of the head and neck region with a fairly good prognosis[6]. Papillary carcinoma is a representative histological type of thyroid malignancy originating from normal follicular cells. Generally, the lesions have mild characteristics and grow only slowly, but cases with certain pathological characteristics are progressive, showing a poor prognosis. Thyroid tumors also account for approximately 1% of all malignant diseases[7]. Previous studies showed that papillary thyroid cancer has a genomic instability[8]. Early detection of the thyroid malignancies reduces the suffering and the cost associated with the disease.

* Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Hacettepe University
** Department of Surgery, Faculty of Medicine, Gazi University Ankara, Turkey
o Corresponding Author: E-mail: uundeger@hacettepe.edu.tr. Tel: +90 312 309 29 58 Fax: +90 312 311 47 77

Various biomarkers have been used to determine cellular DNA damage. The single-cell gel electrophoresis, or the Comet assay, is a valuable method for the assessment of DNA strand breakage in a single cell. It is based on the alkaline lysis of labile DNA at sites of damage. The assay is relatively easy to perform and well-suited for population-based studies[9-13].

The studies describing the levels of DNA damage in malignant and benign thyroid tumours are lacking. For this reason; the aim of this study was to investigate the possible DNA damage in peripheral blood lymphocytes from patients with benign and malignant thyroid disorders in comparison to healthy controls by using the comet assay.

Materials and Methods

Chemicals

The chemicals used in these experiments were purchased from the following suppliers: normal melting agarose (NMA) and low melting agarose (LMA) from Boehringer Mannheim (Germany), sodium chloride (NaCL) and sodium hydroxide (NaOH) from Merck Chemicals (Dramstadt, Germany); dimethylsulfoxide (DMSO), ethidium bromide (EtBr), Triton X-100 and phosphate buffered saline (PBS) tablets from Sigma (St. Loui, USA); ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA), N-lauroyl sarcosinate and tris from ICN Biomedicals Inc. (Aurora, Ohio, USA).

Study Population

The study group consisted of 71 patients with benign or malignant thyroid disorders operated between January 2006 and December 2006 at Gazi University Medical Faculty Department of Surgery; Ankara, Turkey. The patients were divided into three groups; first group consisted of benign thyroid disorders (n=45), the second group consisted of patients with papillary thyroid cancer (n=26) and the third group consisted of 30 healthy controls comparable in sex, socio-economic life style, and smoking habits without history of thyroid or other cancers. The preoperative diagnosis of papillary thyroid cancer patients was confirmed by fine needle aspiration (FNA). Patients with a history of chemotherapy, radiotherapy and thyroid or other diseases other than cancer and who

have thyroid disorders in their families are excluded from the study. All of the patients had the euthyroid stage before the operation and underwent total thyroidectomy. All the subjects were provided with a written informed consent before blood samples were drawn from them. The study has been approved by an ethical committee according to the 'Decleration of Helsinki'. Subjects in all three groups were compared in terms of demographic characteristics, smoking habits and DNA damage in peripheral lymphocytes determined by comet assay.

Sample Collection

Two ml of venous blood samples from each subject were drawn into heparinized tubes and were protected from light. All samples were collected in early morning before the operation.

Cell Preperation

Lymphocytes from a 2 ml heparinized whole blood were isolated by Ficoll-Hypaque density gradient centrifugation and washed in PBS[14]. Cell concentrations were adjusted to approximately $2x10^5$ /ml in the buffer. Aliquots of 5-10µl of the cells were suspended in 75µl of low melting point agarose (LMA) for embedding on slides. Cells were checked for viability by trypan blue exclusion.

Slide preparation

The basic alkaline technique of Singh et al., as further described by Collins et al. was followed[15, 16]. The microscopic slides had been covered with 1% NMA at about 45 ºC in Ca^{2+} - and Mg^{2+}- free PBS before the experiment. This layer was used to promote the attachment of the second layer. For the second layer, around 10000 cells mixed with 80 µl of 1% LMA (pH 7.4) were rapidly pipetted onto this slide, spread using a cover slip, and maintained on an ice-cold flat tray for 5 min to solidify. After removal of the cover slip, the slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% sodium sarcosinate, pH 10) with 1% Triton X-100 and 10% DMSO added just before use for a minimum of 1 h at 4 ºC.

Electrophoresis

The slides were removed from the lysing solution, drained and placed in horizontal gel electrophoresis tank side by side, avoiding spaces and with the agarose ends facing each other, nearest the anode. The tank was filled with fresh electrophoresis solution (1 mM Na_2EDTA and 300 mM NaOH, pH 13) to a level approximately 0.25 cm above the slides. Before electrophoresis, the slides were left in the solution for 20 min at 4^0C to allow the unwinding of the DNA and expression of alkali labile damage. Electrophoresis was conducted at a low temperature (4 ºC) for 20 min using 24 V and adjusting the current to 300 mA by raising or lowering the buffer level and using a compact power supply (Power Pack P 25 Biometra Analytic GmbH). All of these steps were conducted under dimmed light to prevent the occurrence of additional damage. After electrophoresis, the slides were taken out of the tank, washed in distilled water. Tris buffer (0.4 M Tris, pH 7.5) was added drop wise and gently to neutralize the excess alkali and the slides were allowed to sit for 5 min. The neutralizing procedure was repeated 3 times.

Staining and slide scoring

To each slide, 30 µl of EtBr (20 µg/ml) was added. For visualization of DNA damage, slides were examined at a 1000X magnification using a 40X objective on a fluorescence microscope Leica (Wetzlar, Germany). Measurements were made by a computer-based image analysis system 'Comet Assay III' Perceptive Instruments (Suffolk, England). Images of 100 randomly selected lymphocytes, i.e. 50 cells from each of two replicate slides were analyzed from each sample and tail length, tail intensity and tail moment were measured on the screen. Breaks in the DNA molecule disturb its complex supercoiling, allowing liberated DNA to migrate towards the anode. Staining shows the DNA as 'comets'. The mean value of the tail length, tail intensity and tail moment was calculated and used for the evaluation of DNA damage.

Statistical Analysis

For statistical analysis SPSS for Windows 10.0 computer program was used. Results were expressed as mean±SE and the statistical comparison of the results from the healthy controls and the patients with benign and malignant thyroidal diseases were performed using one-way

analysis of variance (ANOVA) test and post hoc analysis of group differences was performed by the LSD test. This test was also used to compare the grades of DNA damage in patients and controls according to smoking habits. $p<0.05$ was considered statistically significant.

Results and Discussion

The first group consisted of 31 females and 14 males (n=45) with benign thyroidal diseases with a mean age of 43±9.3 years. In this group of total 45 benign thyroidal diseases, patients dispersed as 30 multinodular goiter and 15 thyroiditis. All of them have euthyroid state before the operation. The smoker/non-smoker ratio was 11/45. The second group consisted of 18 females and 8 males (n=26) with papillary thyroid cancer with a mean age of 46±13.9 years. All of them have euthyroid state before the operation. The smoker/non-smoker ratio was 4/26. All the patients with benign and malignant group underwent total thyroidectomy. The third group consisted of 30 healthy controls; 10 male, 20 female with a mean age of 29±5.9 years. The smoker/non-smoker ratio was 9/30 in this group.

The DNA damage expressed as tail length, tail intensity and tail moment in the lymphocytes of patients with benign thyroid tumours and thyroid cancer and also healthy controls was given in Table I. The DNA

TABLE I

DNA damage in the healthy controls, patients with bening and malignant thyroid tumours expressed as tail length, tail intensity and tail moment.

Lymphocytes	Tail length	Tail intensity	Tail moment
Healthy controls	0.66±0.01	3.09±0.20	0.02±0.00
Patients with bening thyroid tumours	0.93±0.03 ***	5.98±0.71 **	0.06±0.01 ***
Patients with malignant thyroid tumours	0.85±0.04 ***	5.68±0.71 *	0.04±0.01 *

The results are given as mean±SE.
*** $p<0.001$, ** $p<0.01$, * $p<0.05$ patients with bening and malignant thyroid tumours compared to healthy controls.

damage was significantly higher in patients with thyroid cancer and benign thyroid tumours compared to healthy controls. Although the tail length, tail intensity and tail moment were found to be higher in the peripheral lymphocytes of the patients with benign breast tumors in comparison to breast cancer patients, there were no statistically significant differences in terms of tail length, tail intensity, and tail moment between the patients with breast cancer and benign breast tumors (Figure 1). Cigarette smoking was not related to DNA damage observed in the lymphocytes of the patients with benign thyroid tumours and thyroid cancer, since no additional DNA damage was observed when the smoking and the non-smoking patients were compared. However, the number of smoking patients with benign thyroidal diseases (n = 11) and thyroid cancer (n = 4) is not adequate to give a correct decision on smoking-induced DNA damage.

Thyroid cancer forms in the thyroid gland, an organ at the base of the throat that modulates hormones to help in control heart rate, blood pressure, body temperature, and weight. Papillary, follicular, medullary, and anaplastic thyroid cancers are the four main types of thyroid cancer. Thyroid tumours are the most common endocrine malignancy, accounting for approximately 1% of all malignant diseases and about 0.4% of deaths related to cancer[17]. Since thyroid cancer shows the fastest rate of increase especially in women, there is an urgent need to better understand its underlying causes, explore efficient and sensitive methods for its diagnosis, new methods of treatment and follow-up[18, 19]. Complex interactions between genetic and environmental factors affect the site of tumor formation and play an important part in early tumorigenesis[20]. Increased genetic instability, either spontaneous or mutagen-induced, has been considered as a predisposing factor for neoplastic transformation [21]. It has been argued that any situation that increases the mutation rate can also accelerate carcinogenesis[22]. A sensitive assay to identify markers that can accurately diagnose the onset of thyroid cancer using non-invasively collected blood samples is ideal for early detection. The earlier and more accurate the diagnostic marker can predict the disease onset, the more valuable it becomes.

In the present study the DNA damage in the peripheral lymphocytes of the patients with benign thyroidal nodules and papillary thyroid carcinomas which is a well differentiated thyroid tumour were compared with healthy control subjects by comet assay. It was found that the DNA

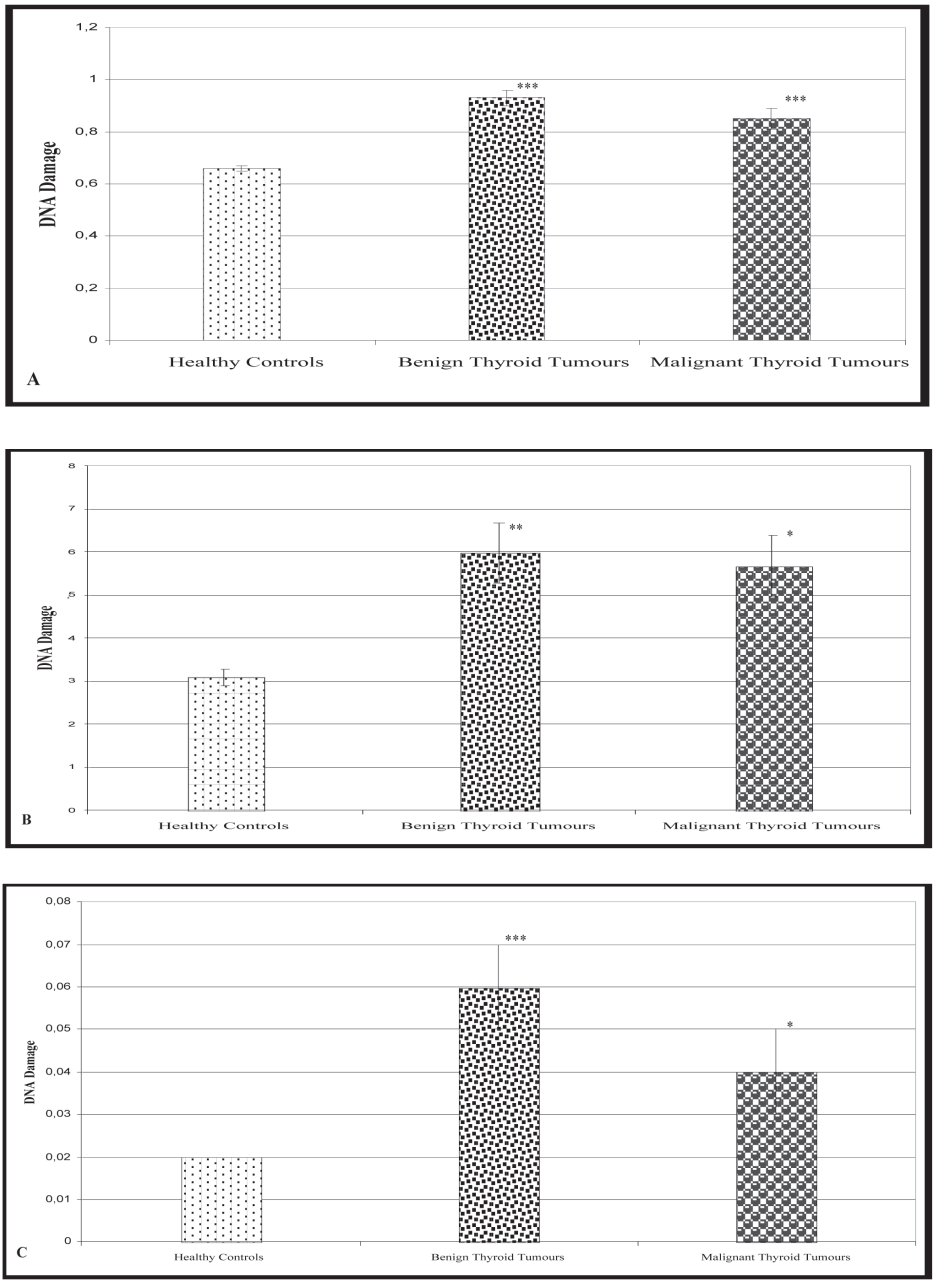

Figure 1

DNA damage in peripheral lymphocytes of the groups expressed as (A) tail length, (B) tail intensity and (C) tail moment (*** $p<0.001$, ** $p<0.01$, *$p<0.05$).

damage in the thyroid cancer patients and in the benign thyroid tumours were higher than the healthy controls. Our findings support the hypothesis that DNA damage may serve as a thyroid cancer risk biomarker. The comet assay used in this study is sensitive to detect significant differences in single strand breaks in benign thyroid tumours and carcinomas. Although there is considerable evidence that individual variation in the detection, signaling, toleration, and repair of DNA damage contributes to human cancer risk. Limited number of studies have reported increased thyroid, breast and bladder cancer risks associated with higher DNA damage measured by the comet assay[15, 23-25]. This is central to the problem of carcinogenesis. DNA damage checkpoint is activated in a wide variety of human preneoplastic and neoplastic lesions. Increased normal cell proliferation, in which case the response of the preneoplastic and neoplastic lesions could simply be a reflection of their high proliferation index. Enhanced proliferative index and DNA damage has been shown in normal colonic mucosa by Gorgoulis et al.[26]. Although it would be ideal to use target tissue in evaluating DNA damage peripheral lymphocytes were used in our study as by Smith et al. since it is easy and rapid to work[24].

In the present study, although the difference is not statistically significant, the DNA damage seems to be higher in the patients with benign thyroid tumours compared to the patients with thyroid cancer. DNA repair capacity is essential for cell survival and the maintenance of cell cycle control. Inter-individual variation in DNA repair capacity has been observed in several in vitro lymphocyte assays[27, 28]. In addition to all these several studies have proven that the difference between DNA damage and repair capacity leads to genetic differences inducing susceptibility to carcinogenesis has been shown in many studies. Popanda et al. reported that deficiency in DNA repair is a risk factor for the development of breast cancer; Wu et al. reported that the DNA repair capacity of lung cancer patients was significantly lower than that of controls; Udumudi et al. found that low DNA repair capacity is a susceptibility factor for cervical carcinoma[29-31]. Recent studies have shown the DNA damage acts as an anticancer barrier. DNA damage response may serve as an inducible barrier to constrain tumour development in its early, pre-malignant stages and create environment that, over time, selects for mutations in checkpoint genes. DNA damage response may rescue defective cell growth, avoid senescence and limit cell death at the expense of genomic instability and tumour progression[26, 32-35]. Thus, activation of the DNA damage check-

point occurs specifically in preneoplastic and neoplastic lesions. Some benign proliferative lesions, both with and without atypia, seem to contain precursor mutations that might affect neoplastic potential[36, 37]. This concept has been formulated, based on the results obtained from analyses of human solid tumours derived from somatic cells, including the various stages of lung, urinary bladder, colorectal, breast cancer and melanomas, as well as some mouse tumour models and cultured human somatic cells[26, 33-35, 38].

Conclusion

To assist with early detection/prevention of papillary thyroid cancer, reliable risk biomarkers are urgently needed. Our results support the hypothesis that increased thyroid cancer risk is associated with higher DNA damage and evaluation of DNA damage response may contribute to early detection and prevention of thyroid cancer but due to enhanced proliferative index in certain benign disorders, use of DNA damage only may not be sufficient to show risk of progression to cancer. Studies with larger population and also in other cells apart from lymphocytes are necessary. On the other hand; our results also show that comet assay seems to be reliable, rapid, and sensitive method for detecting DNA damage in individual cells and fulfils the requirements of a biological marker to detect/prevent thyroid cancer risk.

Acknowledgement

This study was supported by Gazi University, Scientific Project Unit (01/2007-71).

Summary

Assessment of DNA Damage in the Peripheral Blood Lymphocytes From Patients with Benign and Malignant Thyroid Disorders

Thyroid tumours are the most common endocrine malignancy, accounting for approximately 1% of all malignant diseases and about 0.4% of deaths related to cancer. Several studies have reported increased thyroid, breast and bladder cancer risks associated with higher DNA damage measured by the comet assay. The aim of the present study was to

investigate the possible DNA damage in the peripheral blood lymphocytes patients with benign and malignant thyroid disorders in comparison to healthy controls by using the comet assay. In this study, DNA damage expressed as tail length, tail intensity and tail moment of 45 patients with benign and 26 patients with malignant thyroid disorders operated in Gazi University, Medical Faculty, Department of Surgery were compared with 30 healthy controls comparable in sex, socio-economic life style, and smoking habits with no history of any thyroid disease. Patients with benign and malignant thyroid disorders were not received chemotherapy and radiotherapy. Significant increases were observed in the DNA damage in patients with benign and malignant thyroid disorders compared to the healthy controls. No significant differences were found in the DNA damage between benign and malignant thyroid disorders.

Key words: Thyroid tumours, Thyroid cancer, DNA damage, Comet assay, Single cell gel electrophoresis.

Özet

Benign ve Malign Tiroid Hastalarının Periferal Kan Lenfositlerinde DNA Hasarının Değerlendirilmesi

Tiroid tümörleri en sık görülen malign endokrin hastalıklar olup malign hastalıkların yaklaşık %1'ini ve kansere bağlı ölümlerin ise %0.4'ünü oluşturmaktadır. Çok sayıda çalışmada tiroid, meme ve mesane kanser riskinin comet yöntemiyle saptanan artmış DNA hasarıyla ilişkilendirildiği bildirilmiştir. Çalışmanın amacı, benign ve malign tiroid hastalığı olan bireylerin peripheral kan lenfositlerindeki olası DNA hasarını bilgisayarlı analiz sistemli comet yöntemiyle tayin etmek ve sonuçları sağlıklı kontrol bireylerindeki DNA hasarıyla karşılaştırmaktır. Bu çalışmada; Gazi Üniversitesi, Tıp Fakültesi, Genel Cerrahi Anabilim Dalı'nda operasyonu gerçekleştirilen 45 benign ve 26 malign tiroid hastasındaki DNA hasarı kuyruk uzunluğu, kuyruk yoğunluğu ve kuyruk momenti olarak değerlendirilmiş ve sonuçlar cinsiyet, sosyo-ekonomik yaşam tarzı, sigara alışkanlıkları eşleştirilmiş, tiroid hastalığı hikayesi bulunmayan 30 sağlıklı kontrolden elde edilen bulgularla karşılaştırılmıştır. Benign ve malign tiroid hastaları kemoterapi ve radyoterapiye başlamamış hastalardan seçilmiştir. DNA hasarı, benign ve malign tiroid hastalarında

sağlıklı kontrollere kıyasla önemli derecede artmış bulunmuştur. Benign ve malign tiroid hastaları arasında DNA hasarı farklı bulunmamıştır.

Anahtar Kelimeler: Tiroid tümörleri, tiroid kanseri, DNA hasarı, Comet yöntemi, Tek hücre jel elektroforezi.

REFERENCES

1. Bartek, J., Lukas, C., Lukas, J.: Checking on DNA damage in S phase. Nature Reviews in Mol. Cell Biol., 5, 792-804 (2004)
2. Kastan, M., Bartek, J.: Cell cycle checkpoints and cancer. Nature, 432, 316-323 (2004)
3. Bartek, J., Lukas, J.: Pathways governing G1 / S transition and their response to DNA damage. FEBS Lett., 490, 117-122 (2001)
4. Bartek, J., Lukas, J.: The Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell, 3, 421-429 (2003)
5. Lukas, J., Lukas, C., Bartek, J.: Mammalian cell cycle checkpoints: signaling pathways and their organization in space and time. DNA Repair, 3; 997-1007 (2004)
6. Hiroshimaya, T., Takahashi, H., Yao, K., Inagi, K., Nakayama, M., Makoshi, T., Nagai, H., Okamoto, M.: Clinical review of thyroid malignant tumour. Acta Otolaryngol., 547, 85-87 (2002)
7. Ito, Y., Hirokawa, M., Jikuzono, T., Takamura, Y., Miya, A., Kobayashi, K., Matsuzuka, F., Kuma, K., Miyauchi, A.: Extranodal Tumour Extension to Adjacent Organs Predicts a Worse Cause-specific Survival in Patients with Papillary Thyroid Carcinoma. World J. Surg., 31, 1194-1201 (2007)
8. Rigual, N.R., Anderson, G.R., Loree, T.R., Wiseman, S., Alrawi, S., Stoler, D.L.: Molecular prognosticators and genomic instability in papillary thyroid cancer. Laryngoscope, 115, 1479-1485 (2005)
9. Calderon-Garciduenas, L., Osnaya, N., Rodriguez-Alcaraz, A., Villareal-Calderon, A.: DNA damage in nasal respiratory epithelium from children exposed to urban pollution. Environ. Mol. Mutagen., 30, 11-20 (1997)
10. Andreoli, C., Leopardi, P., Crebelli, R.: Detection of DNA damage in human lymphocytes by alkaline single cell gel electrophoresis after exposure to benzene or benzene metabolites. Mutat. Res., 377, 95-104 (1997)
11. Vodicka, P., Tvordik, T., Osterman-Golkar, S.: An evaluation of styrene genotoxicity using several biomarkers in a 3-year follow-up study of hand-lamination workers. Mutat. Res., 445, 205-224 (1999)
12. Zhu, C.Q., Lam, T.H., Jiang, C.Q., Wei, B.X., Lou, X., Liu, W.W., Lao, X.Q., Chen, Y.H.: Lymphocyte DNA damage in cigarette factory workers measured by the Comet Assay. Mutat. Res., 444, 1-6 (1999)
13. Moller, P., Knudsen, L.E., Loft, S., Wallin, H.: The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. Cancer Epidem. Biomar. Prev., 9, 1005-1015 (2000)

14. Boyum, A.: Isolation of lymphocytes, granulocytes, and macrophages. Scand. J. Immunol., 5, 9-15, (1976)

15. Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L.: A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res., 175, 184-191 (1988)

16. Collins, A.R., Dusinska, M., Franklin, M., Somorovska, M., Petrovska, H., Fillion, L., Panayiotidis, Ö., Raslova, K., Vaughan, N. : Comet assay in human biomonitoring studies: reliability, validation and applications. Environ. Mol. Mutagen., 30, 139-146 (1997)

17. Trimboli, P., Ulisse, S., Graziano, F.M., Marzullo, A., Ruggieri, M., Calvanese, A., Piccirilli, F., Cavaliere, R., Fumarola, A., DaArmiento, M.: Trend in Thyroid Carcinoma Size, Age at Diagnosis, and Histology in a Retrospective Study of 500 Cases Diagnosed Over 20 Years. Thyroid, 16; 1151-1155 (2006)

18. Molecular basis of thyroid cancer. NR Farid editor. Amsterdam: Kluwer Academic Publishers, (2004)

19. Hayat, M.J., Howlader, N., Reichman, M.E., Edwards, B.K.: Cancer statistics, trends and multiple primary cancer analyses from the surveillance, epidemiology, and end results (SEER) program. The Oncologist, 12, 20-37 (2007)

20. Ellsworth, D.L., Ellsworth, R.E., Liebman, M.N., Hooke, J.A., Shriver, C.D.: Genomic instability in histologically normal breast tissues: implications for carcinogenesis. Lancet Oncol., 5, 753-758 (2004)

21. Zheng, Y.L., Loffredo, C.A., Yu, Z., Jones, R.T., Krasna, M.J., Alberg, A.J., Yung, R., Perlmutter, D., Enewold, L., Harris, C.C., Shields, P.G.: Bleomycin-induced chromosome breaks as a risk marker for lung cancer: a case-control study with population and hospital controls. Carcinogenesis, 24, 269-274 (2003)

22. Venkitaraman, A.R.: Preface: Chromosomal instability and breast cancer pathogenesis. J. Mammary Gland Biol. & Neoplasia, 9, 217-220 (2004)

23. Sigurdson, A.J., Hauptmann, M., Alexander, B.H., Doody, M.M., Thomas, C.B., Struewig, J.P., Jones, I.M.: DNA damage among thyroidcancer and multiple cancer cases, controls, and long-lived individuals. Mutat. Res., 586, 173-188 (2005)

24. Smith, T.R., Miller, M.S., Lohman, K.K., Case, L.D., Hu, J.J.: DNA damage and breast cancer risk. Carcinogenesis, 24, 883-889 (2003)

25. Schabath, M.B., Spitz, M.R., Grossmann, H.B., Zhang, K., Dinney, C.P., Zheng, D.J., Wu, X.: Genetic instability in bladder cancer assessed by the comet assay. J. Natl. Cancer Inst., 95, 540-547 (2003)

26. Georgoulis, V.G., Vassiliou, L.V., Karakaidos, P., Zacharias, P., Kotsiras, A., Likoğlu, T.: Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature, 434, 907-913 (2005)

27. Mohrenweiser, H.W., Jones, I.M.: Variation in DNA repair is a factor in cancer susceptibility: a paradigm for the promises and perils of individual and population risk estimation? Mutat. Res., 400, 15-24 (1998)

28. Schmezer, P., Rajaee-Behbahani, N., Risch, A., Thiel, S., Rittgen, W., Drings, P., Dienemann, H., Kayser, K.W., Schulz, V., Bartsch, H.: Rapid screening assay for mutagen sensitivity and DNA repair capacity in human peripheral blood lymphocytes. Mutagenesis, 16, 25-30 (2001)

29. Popanda, O., Ebbeler, R., Twardella, D., Helmbold, I., Gotzes, F., Schmezer, P., Thielmann, H.W., von Fournier D., Haase, W., Sautter-Bihl, M.L., Wenz, F., Bartsch, H., Chang-Claude, J.: Radiation-induced DNA damage and repair in lymphocytes from breast cancer patients and their correlation with acute skin reactions to radiotherapy. Int. J. Radiat. Oncol. Biol. Phys., 55, 1216-1225 (2003)

30. Wu, X., Zhao, H., Wie, Q., Amos, C.I., Zhang, K., Guo, Z., Qiao, Y., Hong, W.K., Spitz, M.R.: XPA polymorphism associated with reduced lung cancer risk and a modulating effect on nucleotide excision repair capacity. Carcinogenesis, 24, 505-509 (2003)
31. Udumudi, A., Jaiswal, M., Rajeswari, N., Desai, N., Jain, S., Balakrishna, N., Rao, K.V., Ahuja, Y.R.: Risk assessment in cervical dysplasia patients by single cell gel electrophoresis assay: a study of DNA damage and repair. Mutat. Res., 412, 195-205 (1998)
32. Blasiak, J., Arabski, M., Krupa, R., Wozniak, K., Rykala, J., Kolacinska, A., Morawiec, Z., Drzewoski, J., Zadrozny, M.: Basal, oxidative and alkylative DNA damage, DNA repair efficacy and mutagen sensitivity in breast cancer. Mutat. Res., 554, 139-148 (2004)
33. Bartkova, J., Horejsi, Z., Koed, K., Krämer, A., Tort, F., Zieger, K., Guldberg, P., Sehested, M., Nesland, J.M., Lukas, C., Ørntoft, T., Lukas, J., Bartek, J.: DNA damage response as a candidate anti-cancer barrier in early human tumourigenesis. Nature, 434, 864-870 (2005)
34. Bartkova, J., Rezaei, N., Liontos, M., Karakaidos, P., Kletsas, D., Issaeva, N., Vassiliou, L.V., Kolettas, E., Niforou, K., Zoumpourlis, V.C., Takaoka, M., Nakagawa, H., Tort, F., Fugger, K., Johansson, F., Sehested, M., Andersen, C.L., Dyrskjot, L., Ørntoft, T., Lukas, J., Kittas, C., Helleday, T., Halazonetis, T.D., Bartek, J., Gorgoulis, V.G.: Oncogene-induced senescence is part of the tumourigenesis barrier imposed by DNA damage checkpoints. Nature, 444, 633-637 (2006)
35. Di Micco, R., Fumagalli, M., Cicalese, A., Piccinin, S., Gasparini, P., Luise, C., Schurra, C., Garre, M., Nuciforo, P.G., Bensimon, A., Maestro, R., Pelicci, P.G., d'Adda di Fagagna, F.: Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature, 444, 638-642 (2006)
36. Kaneko, M., Arihiro, K., Takeshima, Y., Fujii, S., Inai, K.: Loss of heterozygosity and microsatellite instability in epithelial hyperplasia of the breast. J. Exp. Ther. Oncol., 2, 9-18 (2002)
37. Euhus, D.M., Cler, L., Shivapurkar, N., Milchgrub, S., Peters, G.N., Leitch, A.M., Heda, S., Gazdar, A.F.: Loss of heterozygosity in benign breast epithelium in relation to breast cancer risk. J. Natl. Cancer Inst., 94, 858-860 (2002)
38. DiTullio, R.A., Jr Mochan, T.A., Venere, M., Bartkova, J., Sehested, M., Bartek, J., Halazonetis, T.D.: 53BP1 functions in an ATM-dependent checkpoint pathway that is constitutively activated in human cancer. Nature Cell Biol., 4, 998-1002 (2002)