Biomarkers, 2011; 16(8): 718–719

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2011.629059

informa
healthcare

LETTER TO THE EDITOR

Increase in serum protein carbonyl groups is associated with more advanced stage of disease in multiple myeloma patients

Dear Sir,

Increases in the generation of reactive oxygen species (ROS) and decreases in antioxidant enzyme activities have been reported in several diseases (Minelli et al. 2009). ROS and free radicals are in fact known to be the mediators of phenotypic and genotypic changes that lead from mutation to neoplasia. However, previous studies have provided conflicting evidence on the association between protein oxidation and cancer risk, with positive results for Hodgkin's lymphoma and bladder cancer, breast cancer and colorectal cancer but not for lung or brain cancer (Morabito et al. 2004; Kumar et al. 2009; Mannello et al. 2009; Yeh et al. 2010).

The action of reactive oxygen species on proteins has been widely demonstrated to increase the formation of carbonyl groups (CG) (Dalle-Donne et al. 2003). In this study, we measured the serum concentration of protein CG in order to quantify the oxidative stress in patients with multiple myeloma (MM) patients.

The study was conducted on 21 MM subjects. Subjects were newly diagnosed patients (12 F–9 M) with a median age of 67 ± 9 years. According to the Durie–Salmon staging system, 6 patients had MM disease stage I, 6 patients had stage II, and 9 patients had disease stage III. The paraprotein class was immunoglobulin IgG in 11 patients and IgA in 9 patients. 1 patient had non-secretory disease. 14 subjects had lytic bone disease and/or pathologic fractures.

Each MM patient was described by complete blood count, liver and renal function tests, immunoglobulins (Ig), lactate dehydrogenase (LDH), β 2 microglobulin level (β2 m). Physical examination, and bone X-ray were performed in all instances.

None of the patients were receiving chemotherapy or anti-inflammatory drugs.

Sera from 30 gender-matched and age-matched normal subjects were also included. None of the patients or of control subjects had active infections, inflammatory diseases, diabetes, hypertension or kidney failure.

Address for Correspondence: Sebastiano Gangemi, University of Messina, AOU Policlinico Via Consolare Valeria 98125, Messina, Italy. Phone: 00390902212075. Fax: 0039090694773. E-mail: sebastiano.gangemi@unime.it

(Received 08 September 2011; revised 28 September 2011; accepted 29 September 2011)

The study was conducted according to the Declaration of Helsinki and it was approved by the local Ethics Committee. Informed written consent was obtained.

The serum content of protein CG was evaluated with use of the Levine method; 100 μL of serum was incubated with 100 μL of a 20 mM 2,4-dinitro-phenylhydrazine solution for 60 min. Then the proteins were precipitated from the solution with the use of 20% trichloroacetate; the protein pellet was washed three times with ethanol and ethyl acetate and resuspended in 1 mL of 6 M guanidine at 37°C for 15 min. The carbonyl content was determined from the absorbance at 366 nm (molar absorption coefficient, 22,000 M^{-1}/cm). The serum concentration of protein carbonyl groups was normalized to the total protein amount determined by the Bradford assay (Sigma-Aldrich, Milan, Italy), and results were expressed as nanomoles of carbonyl groups for protein mg.

The statistical analysis was performed with SPSS for Windows (version 17.0). Data were presented as mean ± standard deviation (SD). Differences between two data series were analyzed by the Mann–Whitney test, differences between more two data series were analyzed by the Kruskal–Wallis test. The correlation between data was verified by a Spearman correlation analysis. Statistical significance was set at $p < 0.05$.

Serum levels of CG were significantly increased in MM patients in comparison to controls (1.340 ± 0.678 nmol/mg proteins vs. 0.650 ± 0.570; $p < 0.001$) (Figure 1A). We observed an increase, in serum levels of carbonylated proteins in MM patients with the more advanced stage of disease. Particularly the difference was more relevant between stage I and II (0.75 ± 0.34 vs. 1.48 ± 0.43; $p = 0.02$) and between I and III (0.75 ± 0.34 vs. 1.48 ± 0.73; $p = 0.05$), while no difference was found between stage II and III (1.48 ± 0.43 vs. 1.48 ± 0.73; $p = 0.82$) (Figure 1B).

There was no correlation between serum levels of carbonylated proteins and classical markers for MM (Hb, β2 m, LDH, Ig, Creatinine, and bone lesions).

Multiple myeloma, is a B-cell malignancy characterised by the accumulation of clonal population of plasma cells, and oxidative stress it has been implicated in carcinogenesis. Accumulation of oxidation products leads to genomic instability, and increasing evidence indicates that oxidative stress play an important role in cell proliferation and participates in cell signaling regulation. Mitogen-activated protein kinase family members, extracellular-regulated kinase, Jun N-terminal kinase, and p38 respond to oxidative stress for their activation. Transcription factors nuclear factor-kappa B and activating protein 1 are also activated by oxidative stress (Gopalakrishna & Jaken 2000).

Previous works have pointed out a strong relationship between oxidative stress and Chronic Myeloid Leukemia

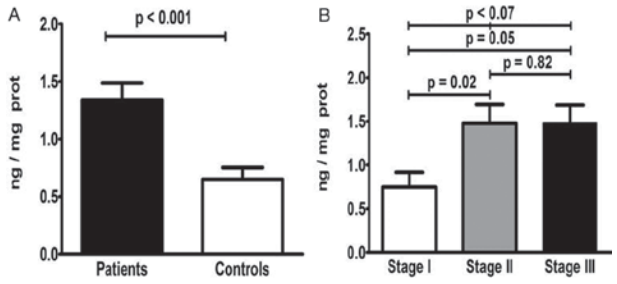

Figure 1. (A) Carbonyl groups serum levels in controls and MM subjects (*$p < 0.001$ vs. controls). (B) Serum levels of protein carbonyl groups in different stages of disease.

and lymphoproliferative disorders (Singh et al. 2009; Zelen et al. 2010), however to our knowledge, data on blood protein CG in MM are not currently available.

Our results showed that carbonyl groups are significantly higher in MM patients as compared to healthy volunteers and we have evidenced an increase of CG in the more advanced stage of disease. These data could be relevant in the onset and in the progression of this disease.

Certain transcription factors exist in fact in a latent state that may be disrupted by oxidative modifications that activate their transcription potential (Doyle & Fitzpatrick 2010). Moreover novel pathways have been identified, such as nuclear respiratory factor-2 (NRF2), and impaired NRF2 function leads to TLR and NFk B activation and this can result in cancer progression.

Further investigation might provide an insight to understand if the increased concentrations of circulating protein carbonyl group is just a consequence of an higher oxidative stress status or if there is a causal link between oxidative stress and MM onset and progression.

Caterina Musolino, Andrea Alonci, and Alessandro Allegra
Division of Hematology, University of Messina, Messina, Italy

Antonella Saija
Department Farmaco-Biologico, School of Pharmacy, University of Messina, Messina, Italy

Giuseppa Penna and Antonino Cannavò
Division of Hematology, University of Messina, Messina, Italy

Mariateresa Cristani
Department Farmaco-Biologico, School of Pharmacy, University of Messina, Messina, Italy

Salvatore Saitta
School and Division of Allergy and Clinical Immunology, Department of Human Pathology, University of Messina, Messina, Italy

Sebastiano Gangemi
School and Division of Allergy and Clinical Immunology, Department of Human Pathology, University of Messina, Messina, Italy and Institute of Biomedicine and Molecular Immunology "A. Monroy" (IBIM), Consiglio Nazionale delle Ricerche (CNR), Palermo, Italy

Declaration of interest

The authors report no declarations of interest.

References

Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. (2003). Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta* 329:23–38.

Doyle K, Fitzpatrick FA. (2010). Redox signaling, alkylation (carbonylation) of conserved cysteines inactivates class I histone deacetylases 1, 2, and 3 and antagonizes their transcriptional repressor function. *J Biol Chem* 285:17417–17424.

Gopalakrishna R, Jaken S. (2000). Protein kinase C signaling and oxidative stress. *Free Radic Biol Med* 28:1349–1361.

Kumar P, Devi U, Ali S, Upadhya R, Pillai S, Raja A, Rao S, Rao A. (2009). Plasma protein oxidation in patients with brain tumors. *Neurol Res* 31:270–273.

Mannello F, Tonti GA, Medda V. (2009). Protein oxidation in breast microenvironment: Nipple aspirate fluid collected from breast cancer women contains increased protein carbonyl concentration. *Cell Oncol* 31:383–392.

Minelli A, Bellezza I, Conte C, Culig Z. (2009). Oxidative stress-related aging: A role for prostate cancer? *Biochim Biophys Acta* 1795:83–91.

Morabito F, Cristani M, Saija A, Stelitano C, Callea V, Tomaino A, Minciullo PL, Gangemi S. (2004). Lipid peroxidation and protein oxidation in patients affected by Hodgkin's lymphoma. *Mediators Inflamm* 13:381–383.

Singh RK, Tripathi AK, Tripathi P, Singh S, Singh R, Ahmad R. (2009). Studies on biomarkers for oxidative stress in patients with chronic myeloid leukemia. *Hematol Oncol Stem Cell Ther* 2:285–288.

Yeh CC, Lai CY, Hsieh LL, Tang R, Wu FY, Sung FC. (2010). Protein carbonyl levels, glutathione S-transferase polymorphisms and risk of colorectal cancer. *Carcinogenesis* 31: 228–233.

Zelen I, Djurdjevic P, Popovic S, Stojanovic M, Jakovljevic V, Radivojevic S, Baskic D, Arsenijevic N. (2010). Antioxidant enzymes activities and plasma levels of oxidative stress markers in B-chronic lymphocytic leukemia patients. *J BUON* 15:330–336.