

ORIGINAL RESEARCH

Medicine Science 2019;8(4):836-40

Examination of serum oxidant-antioxidant levels of workers at dyehouses of textile sector in Denizli's industrial estate**Naci Omer Alayunt¹, Sercan Tasgin²**¹Usak University, Banaz Vocational School, Laboratory Technology Program, Usak, Turkey²Usak University, Department of Occupational Health and Safety, Usak, Turkey

Received 05 August 2019; Accepted 25 September 2019

Available online 24.10.2019 with doi:10.5455/medscience.2019.08.9095

Copyright © 2019 by authors and Medicine Science Publishing Inc.

Abstract

The textile sector is one of the production sectors that contribute greatly to the national economy in domestic and foreign trade. Despite the production of textile products in Turkey, it is still a developing technology-driven human labor. This requires the textile industry to be a business line that must be taken very seriously in terms of occupational health and safety. In this study, it was aimed to determine oxidant-antioxidant parameters and compare with control group in workers in a large textile factory dye house. The control group consisted of 40 people working in the factory or different areas of the factory that had not been exposed to paint and the experimental group consisted of 40 people working in the dye-exposed dye house. From each volunteer 5 ml fasting venous blood sample were taken into yellow cover vacuum plastic gel tube and centrifuged at 4000 rpm for 5-10 minutes and their serums were separated. Vitamins A, E, C, malondialdehyde (MDA), total antioxidant status (TAS) and total oxidant status (TOS) levels were determined by using appropriate kits in high performance liquid chromatography (HPLC) and auto analyser devices in the laboratory and compared with the control group. As a result, it was found that vitamins A, E, C and TAS levels decreased significantly in the dye house compared to the control group ($p < 0.001$) and TOS and MDA levels increased significantly compared to the control group ($p < 0.001$). In the results, the relationship between the control group and the experimental group was examined and evaluated with the help of SPSS program. The findings and results were discussed and suggestions were represented.

Keywords: Antioxidant, malondialdehyde, lipidperoxidation, vitamin, textile dye**Introduction**

The textile sector is one of the sectors that draw the burden of the national economy on domestic trade and export. Textile sector in Turkey still needs the manpower in production and operation phases Despite sophisticated technology [1]. In this respect, the textile sector; It is clear that it is a matter that should be taken very seriously in terms of occupational health and safety. The paint shop employees are also among the professional groups working in this sector.

Dye workers are exposed to certain harmful substances such as aromatic hydrocarbons used as solvents and paint removers. Some of these substances also have clastogenic activity. They form a complex chemical mixture containing well-known genotoxicants such as benzene, toluene and xylene. Thus, chronic occupational

exposure to such substances is thought to cause a genotoxic effect. Known knowledge of genotoxic damage (chromosomal and DNA damage) to workers in the paint is limited, and the knowledge of this damage depends solely on a better understanding of the carcinogenic effect of such substances. Antioxidant systems also serve as biological markers of occupational exposure to genotoxic agents. During normal metabolic reactions in living organisms and radiation, toxic chemicals, drugs, free radicals that are constantly formed by external factors such as antioxidant defense mechanisms are neutralized [2,3]. There are almost no studies on the oxidative effect of dyes and solvents, heavy metals and toxic chemicals that are exposed to workers in the dyeing houses of textile sector. One of the main effects of toxicity from dyes and solvents is the formation of oxidative damage to the cellular system by the release of reactive oxygen species (ROS). The determination of changes in oxidant levels after exposure to solvents, dyes and solvents, which is characterized by MDA and TOS levels, is an important indicator of oxidative damage. Under physiological conditions, free radical production and antioxidant mechanisms are in balance. As a result of the disruption of this equilibrium in the direction

*Corresponding Author: Naci Omer Alayunt, Usak University, Banaz Vocational School, Laboratory Technology Program, Usak, Turkey
E-mail: nacialayunt@hotmail.com

of free radicals, the level of free radical increases and oxidative damage occurs in biological molecules such as lipids, proteins, carbohydrates and DNA [2,4].

In this study, biochemical comparisons were made by focusing on the negative effects of dye in paint shop workers in terms of occupational health and safety, and how oxidant-antioxidant levels were changed as a result of exposure to paint. For this purpose, serum vitamin A, E, C and TAS levels of antioxidant parameters and serum MDA and TOS levels of oxidant parameters were compared.

Material and Methods

The study, working groups, 40 dyehouse workers whose in Denizli textile industry dyeing and consisted of 40 people with an working in unexposed offices or other areas. The mean working time of the experimental group was 13.65 ± 7.98 years. Participants were investigated for factors such as body mass index, smoking, alcohol use, nutritional status, family history of medicine, exposure to electromagnetic fields including cell phones, computers and hair dryers (Table 1).

Table 1. Creating groups

Groups	Group names	Number of people	Agree
Group 1	Control group	n= 40	68.6%
Group 2	Study group	n= 40	46.1%

Study design

Control and study groups were created to make comparisons. The control group consisted of 40 healthy individuals aged between 22 and 59 years who were not exposed to ionizing and non-ionizing radiation for diagnostic and therapeutic purposes. In order to obtain healthy results from the experimental group, blood samples were taken from the individuals who worked for at least 1 year and continued to work. Firat University Non-Interventional Research Ethics Committee approval (Decision No. 05/13 of 28.03.2019), the participants were informed in accordance with the ethical rules and their written permission was obtained.

Laboratory analysis

Serum samples of 5 ml blood samples taken from the experimental and control groups in yellow cover vacuum plastic gel plain biochemistry tubes were separated by centrifugation at 4000 rpm for 5 - 10 minutes and stored at - 20 °C until the working day. Blood samples were taken from the study groups and separated from serum. Serum vitamin A, E, C and MDA levels were analyzed by HPLC (Shimadzu 1700 brand, Japan, (SPD-M20A, RID-20A) pump and UV-Fluorescence absorbance detector) using appropriate mobile phase and standard solutions. In addition, TAS and TOS levels were measured colorimetrically by using Siemens Advia 2400 brand autoanalyser.

Serum vitamin A and E level analysis

Proteins were precipitated by adding 0.3 ml of ethyl alcohol containing 1% H₂SO₄ on 0.3 mL serum sample. The mixture was vortexed and centrifuged at 2500 rpm for 5 minutes. Then 250 µL of n-hexane was added to the samples. By the addition of hexane, the fat soluble vitamins in the medium were extracted to the hexane phase. After hexane was added, it was again mixed

in the vortex and the tubes were centrifuged. At the end of the centrifugation, the hexane phase was carefully separated and taken to the glass tube. 250 µL of n-hexane was added to the sample, mixed and centrifuged, and the n-hexane phase was combined with the hexane phase in the glass tube. The extracted hexane was carefully removed under dry nitrogen. The residue was dissolved in 100 metanoll methanol. It was analyzed by HPLC. In the examples, the E column of inertsil 5µ C-18 (15 cm x 4.6 mm) and the acetonitrile: methanol: dichloromethane: chloroform: hexane (60:10:15:10:5) at the wavelength of vitamin E 296 nm and vitamin A 326 nm in the mobile phase. flow rate 1 mL / min [5].

Serum vitamin C and MDA levels analysis

0.3 mL of the serum sample was taken and 0.3 mL of 0.5 M HClO₄ was added and the proteins were precipitated. This mixture was then vortexed and purified water was added to complete the total volume of 1 mL. After the mixture was centrifuged for 15 minutes (2500 rpm), 20 µL of the clear portion of the samples were carefully analyzed and analyzed by HPLC. Ascorbic acid (vitamin C) and MDA were analyzed by HPLC. Analyzes were carried out using a column of 30 mM C-18 (15 cm x 4.6 mm) at 250 nm in a mixture of 30 mM KH₂PO₄ - methanol (82.5–17.5%; pH: 4) as mobile phase, flow rate in mL / min [6].

Serum TAS and TOS level analysis

Serum TAS and TOS levels were analyzed using a commercial kit. Serum TAS was analyzed at 660 nm (Unit: mmol Torolox. Equiv / L.) using the human kit (Rel Assay Diagnostics brand, ASSAY KIT Catalog no. RL0017 LOT: RL024) and serum TOS was analyzed at 530 nm (Unit: µmol H₂O₂ Equiv./ L.) using the human kit (Rel Assay Diagnostics brand, ASSAY KIT Catalog no. RL0024 LOT: RL026) with measured colorimetrically using Siemens Advia 2400 brand autoanalyser.

Data Analysis

SPSS statistical package program (IBM SPSS Version 22.0) was used to evaluate the data [7]. In this study, Shapiro-Wilk relevance test and homogeneity of variance were checked by “Levene” test and parametric tests were used. Independent Samples T (Student T) test was used to determine the differences between the groups. Data are presented as mean and standard deviation for the groups. Statistical significance level was accepted as p <0.05. In addition, the relationship between the measured parameters was examined by Pearson correlation.

Results

The laboratory findings of the participants were presented in Table 2. The statistical data of serum vitamin A, E, C, MDA, TAS and TOS levels of control and experimental groups are given in Table. There were 40 As exposed workers in study group and 40 workers in control group. When the table is examined, it is seen that there are significant statistical findings between the groups. It has been observed that some blood parameters have changed as a result of occupational exposures of dyehouse employees (Table 2).

There is a balance between free radical production and antioxidant mechanisms under physiological conditions [8]. As a result of the disruption of this balance in the direction of free radicals, the level of free radical increases and oxidative damage occurs in biological molecules such as lipids, proteins, carbohydrates and

DNA. When the data were analyzed, serum vitamin A, E and C levels were decreased at the significance level of $p < 0.001$ in paint shop workers compared to the control group. Serum MDA levels were increased at $p < 0.001$ significance level in paint shop workers compared to the control group. Serum TAS levels were decreased at the significance level of $p < 0.001$ in paint shop workers compared to the control group. Serum TOS levels were increased at the significance level of $p < 0.001$ in paint shop workers compared to the control group (Table 2).

Table 2. Vitamin A, E, C, MDA, TAS and TOS levels

	Control group	Study group	p
Vitamin A (mg/ L)	0.71± 0.14	0.45± 0.06	P< 0.001
Vitamin E (mg/ L)	14.46± 2.24	10.35± 1.74	P< 0.001
Vitamin C (mg/ L)	12.24± 3.84	8.94± 1.51	P< 0.001
MDA (mg/ L)	0.52± 0.18	0.92± 0.25	P< 0.001
TAS (mmol Torolox. Equiv/ L.)	1.94± 0.06	1.56± 0.06	P< 0.001
TOS (µmol H2O2 Equiv./ L.)	7.32± 0.86	10.05± 0.74	P< 0.001

MDA: malondialdehyde; TAS: Total antioxidant status; TOS: Total oxidant status.

*(Mean Standard Deviation) There was a statistically significant difference between the patient and control groups ($p < 0.001$)

Characteristics of participants

Participants were investigated for factors such as body mass index, smoking, alcohol use, nutritional status, family history of medicine, exposure to electromagnetic fields including mobile phones, computers and hairdryers, and are given in the table (Table 3).

Table 3. Experiences of participating groups

Properties of groups	Control group (n= 40)	Study group (n= 40)
Average age	33.35± 10.52	36.75± 7.50
Minimum / maximum age	22- 59	24- 50
Work experience (years)	10.55± 10.19	13.65± 7.98
To smoke	14/ 40	26/ 40
Body mass index (kg/m2)	26.65± 4.16	26.21± 4.67
Body temperature measurements (°C)	36.73± 5.14	36.92± 5.61
Fatigue anxiety and headache	16/ 40	34/ 40
HVETL The average duration of exposure	N/ A	0.53± 0.25
Mobile phone usage time (minutes / month)	537.46± 8.47	504.55± 7.69
Hairdryer (week / week)	1.6± 0.61	1.1± 0.77
Computer use (hours / week)	23.33± 5.61	21.82± 4.22
Ratio of women to men (number of women / number of men)	4/ 36	8/ 32

Note: Data are given as mean ± standard deviation.

Abbreviations: HVETL, high-voltage power transmission lines; N / A does not apply

Discussion

In this study, it was determined that paint sector workers were negatively affected by paints and solvents. The negative effects of factors such as cigarette smoke, exhaust gases, factory fumes, air pollution, noise, excessive exercise on oxidative stress that we are exposed to in daily life have been demonstrated by scientific studies. There are innumerable factors that we may encounter in our business life, which is an important part of our daily life, and that may impair our health. This has led to the idea that there is a need for biochemical studies in occupational health which is a branch of health field. There are almost no studies on the oxidative effects of the dyes and solvents that the textile industry dye workers are exposed to.

Most industrial workers are prone to various diseases due to their exposure to heavy metals and toxic chemicals [9]. One of the main effects of toxicity from dyes and solvents is the formation of oxidative damage to the cellular system by the release of reactive oxygen species (ROS).

It's known that paints, solvents and metals cause DNA damage and oxidative stress [10]. Also, these chemicals and metals can inhibit various enzymes and alter protein conformation [11]. In a study conducted on Brazilian paint workers, it was found that antioxidant enzymes catalase (CAT) and super oxide dismutase (SOD) levels have been found to decrease in paint workers [12]. In the study of Cassini et al., It was said that SOD and CAT activity of paint workers decreased according to exposure time [13].

The duration, age, socioeconomic level, health status and smoking of paint shop workers play an important role in increasing the effect of toxicity. Cigarette smoke contains about 4500 toxic chemicals that combine to form free radicals that cause serious disturbances and play a strong role in inducing oxidative stress [14].

In this study, the data are given as mean and standard deviation instead of statistical evaluation due to the ratio of the number of smokers and non-smokers in the groups (control 14/40, experiment 26/40) (Table 3). Only mean and standard deviation values were calculated; vitamin A, E, C and TAS levels in the control group, respectively; (0.71 ± 0.10 mg/ L) (14.26 ± 2.07 mg/ L); (11.01 ± 2.10 mg/ L); (1.92 ± 0.05 mmol Torolox. Equiv/ L.) and study group (0.45 ± 0.06 mg/ L); (10.04 ± 1.59 mg/ L); (8.70 ± 1.30 mg/ L); (1.56 ± 0.07 mmol Torolox. Equiv/ L.). In the nonsmokers, in the control group, vitamin A, E, C and TAS levels were respectively (0.70 ± 0.16 mg/ L); (14.57 ± 2.40 mg/ L); (12.89 ± 4.45 mg/ L); (1.95 ± 0.06 mmol Torolox. Equiv/ L.) and in the study group (0.45 ± 0.07 mg/ L); (10.91 ± 1.99 mg/ L); (9.36 ± 1.88 mg/ L); (1.55 ± 0.04 mmol Torolox. Equiv/ L.). As a result of laboratory analysis, it was observed that vitamin A, E, C and TAS levels in control and experimental groups were generally lower in smokers compared to non-smokers. Also; MDA and TOS levels in smokers in the control group, respectively; (0.50 ± 0.10 mg/ L); (7.17 ± 0.95 µmol H2O2 Equiv./ L.) and study group (0.95 ± 0.25 mg/ L); (10.13 ± 0.83 µmol H2O2 Equiv./ L.). In non-smokers, MDA and TOS levels in the control group (0.53 ± 0.22 mg/ L); (7.39 ± 0.84 µmol H2O2 Equiv./ L.) and study group (0.86 ± 0.24 mg/ L); (9.9 ± 0.55 µmol H2O2 Equiv./ L.). In the control and study groups, MDA and TOS levels were generally higher in smokers than in non-smokers.

Serum vitamin E and C levels were significantly lower in the experimental group compared to the control group and it can be said that smoking increases this decrease. When the serum oxidant levels were examined, it was seen that non-smokers in the experimental group had lower MDA and TOS values than the smokers. Serum TAS levels in the control group were found to be lower in smokers than non-smokers.

In a study, Duthie et al., Found that serum vitamin C levels in smokers were 50% lower than normal [15]. In our study, serum vitamin C levels were lower in smokers compared to nonsmokers. In a study by Tribble et al., Vitamin C deficiency was found to be 24% in active smokers and 12% in people exposed to passive cigarette smoke compared to non-smokers, and vitamin C levels decreased in proportion to the number of cigarettes smoked [16].

In a study, the mean TOS levels of smokers were reported to be higher than the mean TOS levels of non-smokers and the mean TAS levels in smokers were lower than non-smokers [17]. In this study, TAS levels were higher in non-smokers compared to smokers in the control group, and TOS levels were higher in smokers than non-smokers in the experimental group. Smoking is one of the factors affecting oxidative stress. Cigarette smoke contains many oxidant substances. These oxidants are blamed for the many negative effects of smoking [18]. These components of smoking lead to an increase in oxidative DNA damage and lipid peroxidation through the production of increased reactive oxygen species. Lipid peroxidation causes damage and destruction of cells and intracellular membranes. It can then lead to cell death [19,20].

Antioxidant vitamin levels were found to be lower in workers exposed to paint and smokers than non-smokers. Smoking can increase ROS formation and oxidative stress. Antioxidant enzymes can cause depletion of the body's redox balance.

In the study of Raza et al., it has been reported that workers exposed to paint in the welding, furniture and brick oven industries have an increased tendency to deep cellular damage and oxidative stress [21]. Oxidative damage caused by free radicals can be alleviated by a wide range of antioxidant enzymes and vitamins. It is said that volatile organic compounds increase MDA levels by affecting free radical and antioxidant enzyme activity in textile workers, but do not significantly change TAS levels [22]. Coskun et al. reported that peroxidation induced lipid peroxidation and decreased levels of endogenous antioxidants such as glutathione (GSH), SOD and glutathione peroxidase in the body after exposure to high concentrations of organic solvents [23]. Part of the antioxidant defense systems, which can be endogenous and exogenous, are fat-soluble vitamins (such as vitamins E and A) and water-soluble vitamin C are important antioxidants involved in defending against damage caused by oxidative stress. In the literature, however, few cohort studies of exogenous antioxidants such as vitamin E (α -tocopherol), vitamin A and vitamin C have been found in workers exposed to solvents and paints [24]. In a study, exogenous and endogenous antioxidants were significantly reduced in the paint industry and exposed to paint, while MDA levels, which are indicative of lipid peroxidation, were also increased [22]. In another study, it was reported that the use of the thinner used by paint workers leads to an increase in lipid peroxidation at serum and tissue levels and a decrease in antioxidant vitamins [25,26].

The data obtained in this study support the information of the literature. In the study, it was found that serum oxidant levels (MDA and TOS) of the paint shop workers increased significantly ($p < 0.001$) and antioxidant parameters (Vitamin A, E, C and TAS) were significantly decreased compared to the control group ($P < 0.001$; Table 2). The data obtained in this study showed the presence of lipid peroxidation (LPO) changes in workers exposed to paint and solvent exposure and that the antioxidant system works to reduce the reserve of antioxidant vitamins to eliminate the oxidative stress. Antioxidants have a great effect on LPO in employees, therefore, it should be stated that the evaluation of these antioxidants in paint shop workers may be an important tool in evaluating the imbalance between oxidants and antioxidants in the organism.

Conclusions

As a result, the statistical results of study's data indicate that paint sector workers are exposed to more oxidative stress than office workers. In this study, which is consistent with the literature data, it may be suggested that monitoring of biochemical parameters with oxidative stress biomarkers and antioxidant vitamin supplementation to certain employees in order to prevent accumulation of biological changes due to oxidative stress which may have negative effects on health. In addition, investigating oxidative stress under working conditions in business is a win-win situation for both employers and employees. Adapting health care and counseling for employees can help minimize disease continuity and reduce labor fluctuations.

Conflict of interest

The authors declare that there are no conflicts of interest.

Financial Disclosure

All authors declare no financial support.

Ethical approval

Before the study, permissions were obtained from local ethical committee.

Naci Omer alayunt ORCID: 0000-0003-2215-0934

Sercan Tasgin ORCID: 0000-0002-0338-3233

References

1. Aksu Z. Reactive dye bioaccumulation by *Saccharomyces cerevisiae*. *Process Biochemistry*. 2003;38:1437-44.
2. Cooke MS, Evans MD, Dizdaroglu M, et al. Oxidative DNA damage: Mechanisms, mutation, and disease. *FASEB J*. 2003;17:1195-214.
3. Valko M, Izakovic M, Mazur M, et al. Role of oxygen radicals in DNA damage and cancer incidence. *Mol. Cell. Biochem*, 2004; 266:37-56.
4. Akpoyraz M, Durak I. Biological effects of free radicals. *Ankara J Med*. 1995;48:253-62.
5. Cetinkaya N, Ozcan H. Investigation of seasonal variations in cow serum retinol and beta-carotene by high performance liquid chromatographic method. *Comp Biochem Physiol A Comp Physiol*. 1991;100:1003-8.
6. Karatepe M. Simultaneous Determination of Ascorbic Acid and Free Malondialdehyde in Human Serum by HPLC/UV. *LC-GC North America*. 2004;22:362-5
7. IBM SPSS, IBM Corp. Released. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: USA. 2013.
8. Rock CL, Jacob RA, Bowen PE. Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E, and the carotenoids. *J*

- Am Diet Assoc. 1996; 6:693-702;quiz 703-4.
9. Soleimani E, Moghadam RH, Ranjbar A. Occupational exposure to chemicals and oxidative toxic stress. *Toxicol Environ Health Sci*. 2015;7:1–24.
 10. Liu HH, Lin MH, Liu PC, et al. Health risk assessment by measuring plasma malondialdehyde (MDA), urinary 8- hydroxydeoxyguanosine (8-OH-dG) and DNA strand breakage following metal exposure in foundry workers. *J Hazard Mater*. 2009;170:699–704.
 11. Bulat P, Potkonjak B, Dujic I. Lipid peroxidation and antioxidative enzyme activity in erythrocytes of workers occupationally exposed to aluminium. *Arch Ind Hygiene Toxicol*. 2008;59:81–7.
 12. Moro AM, Charao M, Brucker N, et al. Effects of low-level exposure to xenobiotics present in paints on oxidative stress in workers. *Sci Total Environ*. 2010;408:4461–7.
 13. Cassini C, Calloni C, Bortolini G, et al. Occupational risk assessment of oxidative stress and genotoxicity in workers exposed to paints during a working week. *Int J Occup Med Environ Health*. 2011;24:308–18.
 14. Choi S, Krishnan J, Ruckmani K. Cigarette smoke and related risk factors in neurological disorders: an update. *Biomed Pharmacother*. 2017;85:79–86.
 15. Duthie GG, Arthur JR, Philip W. Effects of smoking and vitamin E on blood antioxidant status. *Am J Clin Nutr*. 1991;53:1061-3.
 16. Tribble DL, J Giuliano LJ, Fortmann SP. Reduced plasma ascorbic acid concentrations in nonsmokers regularly exposed to environmental tobacco smoke. *Am J Clin Nutr*. 1993;58:886-90.
 17. Karademirci M, Kutlu R, Kilinc I. Relationship between smoking and total antioxidant status, total oxidant status, oxidative stress index, vit C, vit E. *Clin Respir J*. 2018;12:2006-12.
 18. Marangon K, Herbeth B, Lecomte E, et al. Diet, antioxidant status, and smoking habits in french men. *Am J Clin Nutr*. 1998;67:231-39.
 19. Naziroglu M, Karaoğlu A, Aksoy AO. Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. *Toxicology*. 2004;195:221–30.
 20. Naziroglu M. New molecular mechanisms on the activation of TRPM2 channels by oxidative stress and ADP-ribose. *Neuroch Res*. 2007;32:1990–2001.
 21. Maryam R, Ishrat M, Muhammad F. Redox balance and DNA fragmentation in arsenic- exposed occupational workers from different industries of Pakistan. *Environmental Science and Pollution Research*. 2018;33:33381–90.
 22. Bayil S, Cicek H, Cimenci IG, et al. How volatile organic compounds affect free radical and antioxidant enzyme activity in textile workers. *Ach Ind Hygiene Toxicol*. 2008;59:283–7.
 23. Coskun O, Oter S, Korkmaz A. The oxidative and morphological effects of high concentration chronic toluene exposure on rat sciatic nerves. *Neurochem Res*. 2005;30:33–8.
 24. Van Loon AJ, Kant IJ, Swaen GM. Occupational exposure to carcinogens and risk of lung cancer: results from The Netherlands cohort study. *Occup Environ Med*. 1997;54:817–24.
 25. Dyatlov VA, Makovetskaya VV, Leonhard R, et al. Vitamin E enhances Cu²⁺ mediated vulnerability of immature cerebellar granule cell to ischemia. *Free Radic. Biol. Med*. 2008;25:793-8002.
 26. Halifeoglu I, Karatas F, Ustundag B, et al. The effect of thinner inhalation on antioxidant vitamins in people working with thinner. *Journal of Biochemistry*. 1999;2:24.