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Comparison of DNA damages in blood lymphocytes of indoor swimming pool lifeguards with non-lifeguards athletes

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ABSTRACT

Chlorination has been used as a major disinfectant process for swimming pool water in many countries. The purpose of this study is to compare the DNA damage of the blood lymphocytes in indoor pool lifeguards with non-lifeguards athletes. We performed a study in which the participants were Gonabad's lifeguards. We chose 30 participants (15 male and 15 female) for each group. We collected vein blood samples from each participant in both exposed and control group. The lymphocytes were isolated from the whole blood by ficoll, and the cell viability was determined by the trypan blue. The alkaline Comet assay was also performed on lymphocytes in order to measure the DNA damage. All the parameters indicated that the DNA damage was significantly greater in lifeguards group than control group ($p < 0.001$). Also, the results revealed a statistically significant higher level of DNA damage in females as evident by an increase in the tail length (μm) [8.97 ± 4.21 for females as compared to 4.32 ± 1.33 for males ($p = 0.001$)], tail DNA (%) [4.18 ± 1.27 for females as compared to 3.14 ± 0.94 for males ($p = 0.016$)] and tail moment (μm) [0.68 ± 0.53 for females and 0.26 ± 0.14 for males ($p = 0.010$)]. There was also a significant positive correlation between DNA damage and the duration of work ($P < 0.001$).

1. Introduction

Chlorination is a common disinfection method for tap water and swimming pool water as it is the most effective and low-cost method compared with others. One of the main uses of chlorine is in disinfection of indoor swimming pools water [1]. Although chlorine is disinfection against almost all kinds of pathogens and it is not expensive to use, it can react with some compounds in the water such as sweat, urine, fat cells and applying cosmetics, which leads to production of some other compounds commonly known as disinfection by-products (DBP) such as Trihalomethane (THM), Ditrichloroaceticacid and 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5 H)-furanone. These compounds have harmful impacts on the body and they can cause DNA damaging. Their carcinogenic potential has been shown in several studies [2]. THMs consist of four compounds: Chloroform ($CHCl_3$), Bromodichloromethane ($CHCl_2Br$), Dibromochloromethane ($CHClBr_2$), and Bromoform ($CHBr_3$). USEPA (1999) reported that these four THMs are human carcinogens of which $CHCl_3$, $CHCl_2Br$ and $CHBr_3$ are carcinogen type B_2 (human carcinogen) and $CHClBr_2$ is carcinogen type C (probable human carcinogen) [3].

People who work in indoor swimming pools are exposed to high doses of Trihalomethane, which is the most important DBP and a potential carcinogen [4], it can penetrate the body through skin permeation, breathing and skin contact [5,6].

A large study in Spain was the first to examine exposure to THMs through ingestion of water and through inhalation and dermal absorption during showering, bathing, and swimming in pools. This study showed that urine mutagenicity increased significantly after swimming, in association with the higher concentration of exhaled bromoform, although no significant associations with changes in micronucleated

Abbreviations: WBC, white blood cell; DNA, deoxyribonucleic acid ; DBP, disinfection by-products; THM, trihalomethane; NMP, normal melting point; LMP, low melting point; EtBr, ethidium bromide; DPD, diethyl-1, 4 Phenylenediamine; PBS, phosphate buffer solution

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urothelial cells were seen [7].

Tap water contains organic matter only from raw water whereas swimming pool water contains that not only from the water but also from swimmers' bodies such as sweat, urine and compounds applied on the skin. These compounds are various nitrogenous compounds such as urea, ammonia and amino acids. Moreover, the water used in some swimming pools is from surface and groundwater. Two factors, organic matter in surface water and bromide ions in ground water, would synergistically generate more brominated-THMs [3]. In a study by Nunn et al, it was proved that 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5 H)-furanone, which is a mutagen for bacteria, is capable of damaging the DNA of human WBCs [8]. Nathaniel et al, who were examining the mutagenic effect of chlorinated and non-chlorinated water on human cells, showed that chlorination may cause a 5.5-fold increase in the mutagenicity of the humic acid-enriched water [9].

As discussed earlier, although swimming in indoor pools is a pleasurable activity, due to exposure of DBPs, it could be a great threat to one's health [10]. Lifeguards are one of the most important staff working in a swimming pool whose duty is to insure the safety of swimmers and saving them if needed. This profession inevitably makes them to spend a considerable amount of their time in the environment of indoor swimming pools and consequently be exposed to DBPs produced as a result of chlorination which causes breathing, skin and eye disorders [11–13]. Since these compounds also have mutagenic and DNA damaging effects and lifeguards are in constant contact with them, we are measuring the degree to which lifeguards are exposed to DBPs through the sensitive and reliable test of Comet assay [14].

2. Materials and methods

2.1. Materials

The chemicals used were normal melting point (NMP) and low melting point (LMP) agarose, along with ethidium bromide (EtBr), all of which were purchased from Boehringer Mannheim. The other chemicals were obtained from MERCK Company.

2.2. Methods

2.2.1. Measurement of chlorine residual

A value for chlorine residual in swimming pools water was obtained by *N*,*N* Diethyl-1,4 Phenylenediamine (DPD) procedure with a Palintest chlorine test kit. Also, air samples were collected to obtain free chlorine content of atmosphere by Methyl Orange Method. Air was collected with a flow sampling pump (flow rate of 1.5 L/min) within 2 m from the water pool and at a height of 1.6 m above the floor level [15].

2.2.2. Subjects of study

This study was conducted on 60 volunteer blood donors in Gonabad city, Iran. Thirty of them were lifeguards that had been occupationally exposed to chlorine and 30 were unexposed control subjects. Since Iran is an Islamic country, pools have different sections for men and women and same numbers of lifeguards are responsible for each section. Inclusion criteria for participants included: being healthy according to medical examinations, willingness to participate in the study, teetotalism, being a non-smoker, not having chewed tobacco, not having consumed alcohol, not having viral illnesses, not having received vaccinations or undergone radiological exams one year prior to the study, not suffering from previous, distinctive, genetic diseases causing DNA damage, and having one year of required the duration of work. However, if a participant were not satisfied with continuing the study, he or she could be excluded from the study. A questionnaire was administered to determine the following parameters: demographic details (age, gender) and personal habits (smoking history, tobacco chewing and alcohol-drinking habits), physical characteristics (height in centimeters and weight in kilograms; a body mass index was calculated from these data, systolic pressure and diastolic pressure), and exposure details (work hours per day and years of exposure duration).

The lifeguards group was selected randomly from the lifeguards list working in the two pools in Gonabad city. Blood samples were collected from subjects who had met the inclusion criteria. The control group was randomly selected from healthy non-lifeguards from university staff with no history of occupational chlorine exposure. They were similar to the lifeguards group in terms of age, gender, and other background variables. To decide on the number of participants needed, a pilot study was carried out on 10 individuals (5 in each group) regarding the average of comet tail % DNA in the two groups and the following results were obtained: $\overline{x_1} = 3.32$, $\overline{x_2} = 2.34$, $S_1 = 1.35$ and $S_2 = 1.09$. By comparing the group means through sample sizes for two independent samples and considering power of the study (0.8) and confidence interval (0.95), the sample size was estimated obtained as 24.57 participants for each group. Having taken account of an attrition rate of 20% for the sample, we chose 30 subjects for each group, coming to 60 participants altogether, all of whom filled in two forms of informed consent and demographic information.

2.2.3. Blood sampling

Blood samples of the exposed and control subjects were collected into heparinized tubes. We collected a whole blood sample in a Heparin tube. Six milliliters of heparinized peripheral blood was gently placed into a tube on the three milliliters of Ficoll-Hypaque (1.077 g/ml) and centrifuged (3300 rpm,20 °C, 15 min) in order to separate the mononuclear cells from whole blood which is mostly consisted of lymphocytes. The supernatant was eliminated and next the pellet was suspended on 500 µl of a phosphate buffer [16]. Comet assay were performed on the lymphocytes. Blood sampling and processing of exposed and control donors were carried out simultaneously. All blood samples were coded, cooled and processed within a maximum of 2 h period after collection. The cell viability was determined by the trypan blue dye- exclusion method [17]. Cell viability higher than 96% was observed. The alkaline Comet assay on lymphocytes was performed immediately after blood transportation.

2.2.4. The comet assay

The Comet assay was carried out under alkaline conditions, basically as described by Singh et al., with minor modifications [18,19]. Fully frosted slides were covered with 1% normal melting point (NMP) agarose. After solidification, the gel was scraped off the slide. The slides were then coated with 0.6% NMP agarose. When this layer had solidified, a second layer containing the suspension of separated lymphocytes within PBS mixed with 0.5% low melting point (LMP) agarose was placed on the slides. After 10 min solidification on ice, the slides were covered with 0.5% LMP agarose. Afterwards the slides were immersed for 1 h in ice-cold freshly prepared lysis solution [2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris–HCl, 1% Na-sarcosinate, pH 10] with 1% Triton X-100 and 10% dimethyl sulfoxide freshly added to lyse cells and were allowed DNA unfolding. The slides were then placed in a horizontal gel electrophoresis tank. The unit was filled with fresh electrophoresis buffer (300 mM NaOH, 1 mM Na_2EDTA, pH $>$ 13) and the slides were set in this alkaline buffer for 20 min to allow DNA unwinding and expression of alkali-labile sites. Denaturation and electrophoresis were performed at 4 °C under dim light. Electrophoresis was carried out for 20 min at 0.65 V/cm and 300 mA to allow the damaged DNA to migrate towards the anode. After electrophoresis the slides were rinsed gently three times with a neutralization buffer (0.4 M Tris–HCl, pH 7.5) to remove excess alkali and then fixed by methanol. Each slide was stained with ethidium bromide (20 µg/ml) for 5 min and finally dipped in ice-cold water to remove the excess ethidium bromide (EtBr) and covered with a coverslip. H_2O_2 with the concentration of 100 µM was used as positive control [20].