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Microbial Analysis and Cytogenetic Effects of Drinking Bottled Water

KEYWORDS: Bottled water, Cytotoxic, PET, PBL

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ABSTRACT *Seventy five samples of bottled water belong to fifteen local and imported brands were collected from grocery shops within Baghdad city from March to June 2012, two variables were investigated, the first one was microbial population diversity represented by fecal coliform, aerobic bacteria and fungi. 10 samples of 75 showed positive results, in failure rate reached to 13.3 %, the highest TVC have been recorded in sample number 3 were 4.6. Fecal coliform showed negative growth in all samples. The second aim of this study was to determine the possible toxicological effects of chemicals released into water packaged in polyethylene terephthalate (PET) bottles by using peripheral blood lymphocytes PBL as a biological system. From the results the mitotic index (MI) and blastogenic index (BI) showed significant reduction ($p \leq 0.05$) when increased the period and temperature of storage accompanied with elevated in chromosomal aberration CA reached to 0.162±0.02 in 9 months of storage and 0.196±0.03 in ≤35°C storage temperature.*

1. Introduction

The water is the key element in the survive of life on earth, in spite of the water covers nearly 75% of the earth's surface, but the proportion of fresh water does not exceed the rate of 1%. However, with the increasing in population density and growth of the agricultural and industrial activities, drinking water has become a scarce commodity in many countries and regions in the world [1]. It is reported that changes in water quality are primarily the result of human activities that would discharge water pollutants or alter water availability [2]. Therefore, the urgent need of drinking water was appeared treated water in different shape; one of these shapes was bottled water in glass or plastic container. The quality and specifications of bottled water depends on the water sources, purification techniques as well as the packing material [3].

Due to the importance of safe drinking water, drinking water is regularly examined to ensure safety in developed countries. It is not practical to monitor the drinking water for every possible pathogen. Therefore, normal intestinal organisms, such as coliform bacteria, are used as indicator of fecal pollution because they are easy to detect and count [4].

For many years it was thought that the mineral and bottled water safer for consumption of tap water, which has strengthened this belief type of treatment which are subjected of this type of water. Whereas several studies suggest that bottled water may have the same or worse bacteriological and chemical quality as tap water, concluding that bottled water is not better than tap water [5,6].

Recently in Iraq very few studies have been carried out to investigate the microbial contamination of bottled water. Therefore, a goal of this study was to determine the current status of bacteriological quality of various brands of bottled water sold in Baghdad city and investigating the diversity of bacterial and fungal. A second goal of this study was to investigate the genotoxic effects of water-packed in plastic containers, on peripheral blood lymphocytes by measuring the mitotic index (MI), balstogenic index (BI) and chromosomal aberrations (CA) in vitro.

2. Materials and methods

Seventy five samples of bottled water belong to fifteen various commercial brands were collected from grocery shops within Baghdad city from March to June 2012. The present work investigated two type of parameters the first one deals with microbial diversity assessment in different size commercial preparations (1.5, 0.5 and 20 liter), all samples were stored in the grocery stores at room temperature, while the other investigation deals with the cytotoxic effects of these water on peripheral blood lymphocytes by determined mitotic index (MI), blastogenic index (BI) and chromosomal aberrations (CA).

For microbial diversity assessment, each bottle was adequately shaken, 2 ml of each sample was taken for microbiological analyses, one milliliter was inoculated in blood agar plate and incubated for 24 hrs at 37°C, quantitative bacteria were determined as total viable count (colony forming unit/ml), while bacterial qualitative was identified depending on cultural and biochemical properties of each colony [7]. Fungal identification was done by inoculating 1 ml of bottled water in Sabouraud dextrose agar plate (SDAP), all plates were incubated for 48 hrs at 25°C, and fungal diagnoses were inspected microscopically [7].

Genotoxic effects of bottled water were examined by determined MI, BI and CA in peripheral blood lymphocytes from healthy people In Vitro, by exposed PBL for three generations to examined water. 100 ml of each sample was concentrated by evaporating in room temperature reaching to 10 ml at sterile conditions, 0.5 ml of concentrated water added to 4 ml of RPMI-1640 supplemented with 15% fetal calf serum, L-glutamine and penicillin and streptomycin. All tubes were incubated at 37°C for 70 hrs. The chromosomal analyses were conducted according to Verma and Babu [8].

3. Results and discussion

3.1 Microbial diversity

Microbial failure rate of bottled water reach to 13.3 %, 10 samples of 75 showed positive results, 4% was displayed fungal growth, represented by Candida albicans in two samples and Cryptococcucs sp in one sample. Bacterial presence appears in 7 specimens represented by Staphylococcus sp in 3

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samples while Pseudomonas sp recurrence of four times. All brands showed negative for fecal coli form test, which is most common group of indicator organisms used in water quality monitoring. These organisms are representative of bacteria normally found in the intestinal tract of mammals including human, so they provide a general, adequate, index of fecal [9,10]. Total viable count (CFU/ml) ranged from 4.6 CFU/ml in sample number 3 to 0.2 CFU/ml in sample number 25 as-shown in Table 1. It can be also note that there is no association between a period of storage and size of container with TVC. The microbial contamination may be due to the type of treatment techniques used in the production or result from bad holding of water by the workers or by contamination of water sources that has not been adequately removed during treated process. Existence microbial contamination after the process of packing may be due to the increase in temperature during storage, and the trace amounts of nutrients in the same water [11].

Table 1: Microbial analysis of bottled water within Baghdad, Iraq

Brand code	Size (L)	Manufacturing date	Expiration date	Fungal content	Total count CFU/ml	Bacterial Content	Total count CFU/ml
2	0.5	26/11/2011	25/11/2012	/	/	Staphylococcus sp.	2.8
3	0.5	15/1/2012	14/1/2013	/	/	Staphylococcus sp.	4.6
7	0.5	28/3/2012	27/3/2013	/	/	Staphylococcus sp.	0.4
25	20	14/8/2011	13/8/2012	/	/	Pseudomonas sp.	0.2
31	20	6/9/2011	5/9/2012	Candida albicans	1.3		
37	20	28/9/2011	27/9/2012	Candida albicans	0.5		
46	20	4/6/2012	3/6/2013	/		Pseudomonas sp.	2.5
55	20	7/12/2011	6/12/2012	Cryptococcucs sp	0.65		
58	20	4/7/2011	3/7/2012	/	/	Pseudomonas sp.	0.44
63	20	5/9/2011	4/9/2012	/	/	Pseudomonas sp.	0.85

3.2. Cytogenetic assessment

The second aim of this study was investigation the effects of PET release in various storage periods and various storage temperatures on PBL which isolated from healthy person, from Table 2 and 3, it can be observed significant reduction in both MI and BI with the increase in storage periods and temperatures accompanied with elevated in CA, represented by chromatid breaks CB, micronuclei MN and ring chromosome RC. The highest proportion of TCA has been observed in the storage period more than 9 month reached to (0.162±0.02), the reduction percent in MI from control to the 9 months of storage period 36.7%, while reduction percent in BI was 25.3%. However, the reduction percentage of MI and BI was 38.34% and 31.74% respectively in PBL exposed to bottle water stored in different temperatures, from Table 3, the results indicated that temperature effects were more evident than storage effects. The results of this study agreed with Evandri et al. [12] who observed an increase in chromosomal aberrations in Allium cepa with PET water samples exposed to direct sunlight for 16 weeks (two-fold induction) and exposed in the dark at 40 °C for 10 days (three-fold induction). Although, these aberrations were attributed to the migration analysis of water was not done. Biscardi et al. [13] performed the Tradescantia micronucleus bioassay and the Comet assay with human leukocytes in lyophilized water stored in PET bottles. Only samples stored for 2 months showed an eight-fold increase in the frequency of micronuclei compared to distilled water [13].

Table 3: Chromosomal analysis of PBL exposed to bottled water stored in PET bottle in different period

Period of storage months	No.	MI	BI	CB	MN	RC	TCA
Control	3	1.93±0.23a	45.67±2.34a	0.0a	0.001± 0.0a	0.0a	0.001±0.0
0-3	25	1.86±0.17a	41.33±1.22b	0.01±0.002b	0.02±0.001b	0.0a	0.03±0.001
3-6	21	1.56±0.22b	40.67±2.56c	0.021±0.01c	0.06±0.01c	0.03±0.002b	0.11±0.002
6-9	18	1.32±0.34c	37.23±2.45d	0.033±0.002d	0.065±0.01d	0.034±0.006b	0.132±0.024
≤ 9	11	1.22±0.12c	34.12±1.29e	0.042±0.06e	0.078±0.02e	0.042±0.005c	0.162±0.02

CB: chromatid break MN: micronuclei RC: ring chromosome TCA: total chromosomal aberration,
Each number represent M±SD for 100 lymphocytes, different letters refer to significant differences at p≤0.05.

Table 4: Chromosomal analysis of PBL exposed to bottled water stored in PET bottle in different temperatures

Temperature of storage °C	No.	MI	BI	CB	MN	RC	TCA
Control	3	1.93±0.23a	45.67±2.34a	0.0a	0.001± 0.0a	0.0a	0.001±0.0
4-10	20	1.91±0.23a	43.21±1.35b	0.011±0.02b	0.03±0.01b	0.0a	0.041±0.01
10-25	20	1.77±0.12b	41.17±1.67c	0.036±0.011c	0.05±0.02c	0.02±0.002b	0.16±0.012
25-35	20	1.54±0.21c	35.23±2.12d	0.046±0.01d	0.065±0.02d	0.043±0.02b	0.145±0.021
≤ 35	15	1.19±0.34d	31.17±1.56e	0.062±0.05e	0.08±0.03e	0.054±0.03c	0.196±0.03

CB: chromatid break MN: micronuclei RC: ring chromosome TCA: total chromosomal aberration,
Each number represent M±SD for 100 lymphocytes, different letters refer to significant differences at p≤0.05.

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REFERENCE

[1] WHO (2011). Guideline for Drinking Water Quality, 4th ed, Geneva, available on the WHO web site http://www.who.int. | [2] Al Obaidy, A.H.M.J. and Al-Khateeb, M. (2013). The challenges of water sustainability in Iraq, Engineering and Technology Journal, Vol. 31, No. 5, pp. 828-840. | [3] Abed, K.F. and Alwakeel, S.S. (2007). Mineral and microbial contents of bottled and tap water in Riyadh, Saudi Arabia, Middle-East Journal of Scientific Research, Vol. 2, No. 3-4, pp. 151-156. | [4] Yousaf, S. and Chaudhry, M.A. (2013). Microbiological quality of bottled water available in Lahore city, Journal of Pakistan Medical Students, Vol. 3, No. 2, pp. 110-112 | [5] Van Der Aa, N.G.F.M. (2003). Classification of mineral water types and comparison with drinking water standards, Environmental Geology, Vol. 44, No. 5, pp. 554–563. | [6] Ahmad, M. and Bajahlan, A. S. (2009). Quality comparison of tap water vs. bottled water in the industrial city of Yanbu (Saudi Arabia), Environmental Monitoring and Assessment, Vol. 159, No. (1-4), pp. 1–14. | [7] Pommerville, J.C. (2010). AlCamo's Fundamentals of Microbiology, 9th ed. Jones and Bartlett Publishers. | [8] Verma, R. and Babu, A. (1989). Human Chromosomes: Manual of Basic Techniques. Pregramon Press, New Yourk. | [9] Fewtrell, L. and Bartram, J. (2001). Water Quality: Guidelines, Standards and Health: Assessment of risk and risk management for water-related infectious disease, Published on behalf of the World Health Organization by IWA Publishing, Alliance House, 12 Caxton Street, London SW1H 0QS, UK. | [10] Stevens, M., Ashbolt, N. and Cunliffe, D. (2003). Review of Coliforms as Microbial Indicators of Drinking Water Quality, Australian Government, National Health and Medical Research Council. | [11] Venieri, D., Vantarakis, A., Komninou, G. and Papapetropoulou, M. (2006). Microbiological evaluation of bottled non-carbonated ("still") water from domestic brands in Greece, International Journal of Food Microbiology, Vol. 107, No. 1, pp. 68–72. | [12] Evandri, M.G., Tucci, P. and Bolle, P. (2000). Toxicological evaluation of commercial mineral water bottled in polyethylene terephthalate: a cytogenetic approach with Allium cepa, Food Additives and Contaminants, Vol. 17, No. 12, pp.1037-1045. | [13] Biscardi, D., Monarca, S., De Fusco, R., Senatore, F., Poli, P., Buschini, A., Rossi, C. and Zani, C. (2003). Evaluation of the migration of mutagens/carcinogens from PET bottles into mineral water by Tradescantia micronuclei test, Comet assay on leukocytes and GC/MS, The Science of the Total Environment, Vol. 302, No. 1-3, pp. 101-108. |