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Dietary Risk Assessment of Acrylamide in School Canteen Snacks among Primary School Students in Alexandria Governorate

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Abstract

BACKGROUND: Acrylamide (AA) is considered as a carcinogenic and genotoxic food contaminant produced in carbohydrate-rich foods. Canteen snacks available in school are among these carbohydrate-rich foods. The paper investigates the risk associated with consuming acrylamide rich canteen snacks.

AIM: The aim of the work was to study the dietary risk of acrylamide in school canteen snacks among primary school students and to determine the concentration of AA in different snacks.

METHODS: The level of AA was detected in 73 samples for 16 different canteen snacks sold at school canteens. Samples were collected randomly from the canteens of primary schools in Alexandria city. All samples were extracted and cleaned-up then analyzed using HPLC techniques. A food frequency questionnaire was designed to determine the frequency and amount of consumption of different canteen snacks among primary school students during school day. The data were collected from six different primary schools in Alexandria city (three public and three private schools). All interviewed school students (466 students) were weighted for the calculation of dietary exposure of AA during school day.

RESULTS: The results showed that the average and the 95th percentile of acrylamide exposure were 1.4 and 4 μgkg^{-1} BWsd^{-1}, respectively. There is a statistically significant difference between dietary exposure to AA between private schools and public schools. The estimated margin of exposure (MOE) calculated from the average acrylamide exposure was 128 and 221 and from 95th percentile was 45 and 77, based on BDML10 values 0.18 mgkg^{-1} BWd^{-1} and 0.31 mgkg^{-1} BWd^{-1}. Some schools offered fried snacks and showed the highest calculated risk of AA exposure.

CONCLUSIONS: Dietary exposure to AA from canteen snacks among primary school students in Alexandria city is considered as a health concern according to the definition of the WHO, where the calculated MOE values were very close to the values set by the JECFA committee (45–310) for health problems of concern.

Introduction

More than 30% of a typical daily calorie intake comes from foods that contain AA. Potato chips, French fries, and other fried foods were found to have the highest possibility of AA formation and the highest levels of AA when compared to other food products [1]. In a recent study conducted at Jeddah schools, the main contributors to the AA exposure from cafeteria foods were cheese pies, chocolate pies, cheese sandwiches, custard pies, and plain cookies. These five items contributed to the AA exposure of primary school students by 66.2%, middle school students by 56.1%, and secondary school students by 69.6% [2].

There is no legal permitted limit for acrylamide level in different food products although the exposure to acrylamide cannot be avoided but the WHO reported that the level of acrylamide in drinking water should not exceed 0.5 μgL^{-1} [3], and the European Commission Regulation (EC 2017/2158) established benchmark levels to reduce the presence of acrylamide in certain food items. The levels set by the EU commission for different food stuff were French fries 500 μg kg^{-1}, potato crisps 750 μg kg^{-1}, biscuits 350 μg kg^{-1} and crisp bread 350 μg kg^{-1} [4].

Various studies have been investigating the carcinogenic effect of AA in humans since its discovery in foods. While AA was found to be carcinogenic in both male and female rodent models, there has been no clear evidence of its carcinogenic effects in humans in the few epidemiologic studies conducted to date on occupational and dietary exposure to AA [5].

Since there are no recommendations for the tolerable daily intake of acrylamide, the margin of exposure approach (MOE) is used to assess the risk of acrylamide in humans [6]. The MOE is described as the lower confidence limit on selected reference point from experimental studies on dose-response data divided by estimated intake of the contaminant. A MOE below 10,000 for AA is commonly regarded as health concern [7]. The JECFA report agreed with the previous conclusions by the EFSA, and the report added also that MOE values between 300 for average acrylamide exposure and 75 for high exposure to acrylamide are considered as public health concern [6].

Acrylamide (AA) intake in children is generally 2–3-fold higher than that of adults, when expressed on a body weight basis [8]. Furthermore, children and adolescents consume more AA rich food, such as potato chips, French fries, fried cereals (crisps, popcorn, or breakfast cereals), crisp bread, cookies, and fine bakery products on a more regular basis than other population [9].

In Canada, they revealed the consumption of deep fried French fries among Canadian teenagers contributed with about 50% of AA exposure followed by potato crisps with 10% then oven baked French fries [10]. In another study, the same pattern was revealed with French fries showing the highest contribution percent (26%) followed by potato crisps (16%) to the overall AA exposure in children and adolescents aged from 7 to 18 years old [11].

Determining the level of AA in various canteen products and dietary exposure of AA from consuming these products among primary school students is crucial. Children are considered to be the most exposed group to AA intake due to their low average body weight and because schoolchildren consume more than one third of their total caloric intake during the school day. To the best of our knowledge, this is the first study to conduct a dietary risk assessment of acrylamide in Egypt.

Materials and Methods

Food sampling

All carbohydrate-rich snacks (a total of 73) were collected school canteens and the dietary consumption data were collected from students at the same time.

Sample preparation procedures

The collected samples were minced using electric mincer then pooled together to give one homogenate sample. Three sub-samples were then used for AA determination. Accurately weighed 1 g of the homogenized sample in 50 mL falcon tubes to each sample 5 mL of HPLC grade hexane was added and shaken for 5 min using a vortex mixer (Fisher Scientific Co., Pittsburgh, PA, USA). The mixture was centrifuged at 10,000 rpm for 15 min in cooling centrifuge; the fat layer was removed after centrifugation. The fat extraction step was repeated until the removal of all fats, sample was then spiked with 100 μL of the MAA internal standard (1 $mgml^{-1}$) and AA was extracted by shaking with 5 mL of water (HPLC grade) for 5 min using a vortex mixer and then centrifuged for 10 min in cooling centrifuge at 10,000 rpm (HISTAM PLUS-RH cooling centrifuge). The supernatant was filtered through 0.2 mm syringe filter and 1 mL of the filtrate was cleaned in pre-conditioned SPE cartridge [2], [12].

Clean-up

The SPE cartridge was conditioned with 5 mL of HPLC grade then adding 5 ml of HPLC grade water. Following the conditioning step, 1 mL of the filtrate was passed through the SPE cartridge. Acrylamide was then eluted with 2 mL of HPLC grade water after leaving the sample in contact with the cartridge for 3 min. The initial drops were discarded, and the filtrate was collected in 1.5 mL amber HPLC vials for chromatographic analysis.

Chromatographic conditions

Reverse-phase high-performance liquid chromatography (HPLC), UltiMate 3000 UHPLC system, Thermo Scientific, USA, equipped with a DIONEX UltiMate 3000 variable wavelength ultraviolet detector (VWD) was used for separation and estimation the level of AA in different food samples collected from school canteens. Separation was performed on analytical column ODS Hypersil C18 (250 × 4.6 mm diameter, 5 micron particle size), Thermo Scientific, USA. The operating conditions were isocratic mobile phase of water and acetonitrile (90:10 v/v). The flow rate was set at 0.6 mL min^{-1} with detection at 195 nm; injection volume, 30 μL; and column temperature 25°C.

Dietary exposure assessment

The frequency of consumption of snacks sold in school canteens was collected from 466 students (258 male and 208 female). The students participating in the study were recruited using a multistage stratified random sampling. The study participants were randomly selected from three private schools and three public schools, from each selected school a class of each grade was selected randomly (i.e., fourth, fifth, and sixth grade). All children of each class were interviewed. A pre-designed food frequency questionnaire (FFQ) [13] was used to determine the frequency and amount of consumption. The food frequency questionnaire included a list of 16 carbohydrate rich snacks. The body weight of each student was measured using an appropriate weight measuring scale with the student in light clothes and no shoes using the procedures of Gibson [14]. The measuring scale was calibrated every day before being used.

The exposure of study sample to AA was determined by combining the level of AA in different snacks with the data on the individual consumption of canteen snacks. Exposure as a result of cafeteria foods was calculated using the formula:

$$Ei = \frac{\Sigma Qik \times Cik}{BWi \times 5}$$

Where, Ei is the dietary AA exposure of student i (μgkg^{-1} BW sd^{-1}), Qik is the amount of food item k consumed by student i over 2 school days, Cik is the AA concentration in food item k (μgkg^{-1}), BWi is the BW of student i (kg), and Σ denotes the sum over all food, items consumed by student i among the cafeteria foods. Thus, the exposure calculated based on school week of canteen snacks consumption) was divided by 5 to obtain the daily AA exposure.

Risk assessment of acrylamide exposure

Margin of exposure (MOE) values were calculated by comparing the mean and 95th percentile values of AA exposure against benchmark dose lower confidence limit (BMDL$_{10}$) values (0.18 mg kg^{-1} BWday^{-1} for the development of Harderian gland tumors in mice and 0.31 mg kg^{-1} BWday^{-1} for the induction of mammary tumors in rats [15].

Statistical analysis

The collected data were analyzed using IBM SPSS software package version 21.0. (Armonk, NY: IBM Crop). Qualitative data were described as number and percent. Quantitative data were described as mean, range (minimum and maximum), standard deviation, median, and percentiles. The dietary intake of AA followed a normal distribution, therefore, independent t-test was used to determine the significance between the mean of two unrelated groups and one-way ANOVA test was used to the determine the significance between the mean of more than 2 unrelated groups followed by *post hoc* test.

Results

Acrylamide levels in different snacks sold in primary school canteens

The level of AA among 16 tested snacks sold in school canteens ranged from 291 to 1889 μgkg^{-1}, as shown in Table 1, the highest level found in potato chips (1889 μgkg^{-1}) followed by French fries (1757 μgkg^{-1}) while the lowest AA levels were found in vanilla cake (298 μgkg^{-1}) followed by strawberry cake 291 μgkg^{-1}. Since potato chips contain the highest AA level among all the snacks analyzed and also the most consumed and most popular snacks among elementary school students, this is the reason that potato chips contributed more than half of the AA exposure (51.92%) followed by French fries (12.41%), as shown in Table 1.

Table 1: Acrylamide levels (μg/kg) in canteen snacks in Alexandria city

Canteen snacks	Acrylamide level in μg/kg			
	Number of samples	Mean ± SD	Minimum–maximum	Contribution (%)
Potato chips	8	1889 ± 98	1777–1960	51.92
French fries	2	1757 ± 122	1640–1883	12.41
Chicken pane	2	1642 ± 107	1530–1743	3.57
Pizza	2	1123 ± 379	740–1497	0.09
Colored crackers	1	854 ± 399	593–1313	0.53*
Toasted bread	9	742 ± 167	557–880	6.74
Corn chips	6	549 ± 22	530–573	5.28
Donuts	1	532 ± 51	493–590	0.53*
Chocolate biscuits	9	462 ± 39	437–507	6.48
Chocolate cake	5	458 ± 150	290–577	3**
Cheese croissant	6	408 ± 39	270–467	2.31
Popcorn	3	374 ± 172	263–573	0.53*
Plain biscuits	6	332 ± 78	243–387	5.99
Chocolate croissant	5	323 ± 107	200–383	1.72
Vanilla cake	4	298 ± 63	237–363	3**
Strawberry cake	4	291 ± 47	277–343	3**

*Others (popcorn-colored crackers-donuts), **cake. SD: Standard deviation.

Dietary exposure of acrylamide from canteen snacks

Table 2 shows that the overall mean exposure and 95th percentile based on the consumption during the school days only were 1.4 and 4 μgkg^{-1} BWsd^{-1}, respectively. The table also illustrates AA consumption in private and public schools, 1.6-1.8 μgkg^{-1} Bwsd^{-1} respectively with a statistical significant difference.

Table 2: Mean acrylamide exposure (μg/kg body weight/SD) from canteen snacks students of different educational levels

Educational levels	Gender	Sample size	Mean	P50	P90	P95	Test of significance (p)
Fourth grade	Boys	91	1.57	1	3.8	3.35	0.158
	Girls	71	1.29	1	3	4	
Fifth grade	Boys	94	1.43	1	3	3	0.247
	Girls	76	1.23	1	3	3.15	
Sixth grade	Boys	73	1.36	1	3	4	0.368
	Girls	60	1.55	1	3	4	
Type of school	Public	215	1.18	1	3	3	> 0.001
	Private	251	1.6	1	3	4	
Total	Boys	258	1.4	1	3	4	0.162
	Girls	208	1.37	1	3	4	
	Total	466	1.4	1	3	4	

**The difference is significant at $\alpha \leq 0.005$.

Risk characterization

The scientific committee of the EFSA stated that the margin of exposure (MOE) approach can be used when assessing the risk of a substance which is genotoxic and carcinogenic in different foods. The results of the present study (Table 3) showed that the overall margin of exposure (MOE) calculated from the mean exposure to AA and based on BMDL$_{10}$ (0.18 mgkg^{-1} BWday^{-1}) was 128 and based on BMDL$_{10}$ (0.31 mgkg^{-1} BWday^{-1}) was 221. For the 95th percentile (high consumption group), the calculated MOEs was 45 based on BMDL$_{10}$ (0.18 mgkg^{-1} BWday^{-1}) and 77 based on BMDL$_{10}$ (0.31 mgkg^{-1} BWday^{-1}).

Discussion

Children are the most vulnerable age group to the possible risks of AA due to their low average

Table 3: Margin of exposure for mean and 95th percentile of acrylamide dietary exposure

MOE	Fourth grade		Fifth grade		Sixth grade		Private schools		Public schools		Total	
	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95	Mean	P95
Dietary exposure	1.44	4	1.34	3	1.45	4	1.6	4	1.18	3	1.4*	4
$BMDL_{10}$=0.18 mg/kg BW/day	125*	45	134	60	124	45	152*	60	112	45	128	45
$BMDL_{10}$=0.31 mg/kg BW/day	215	77	231	103	213	77	262	107	193	77	221	77
$BMDL_{10}$=0.43 mg/kg BW/day	298**	107	320	143	296	107	268	107	364**	143	307**	107

*For carcinogenic and genotoxic effect, MOE below 10,000 would be of public health concern, **For non-genotoxic effect (neurotoxic), MOE above 100 would be of no public health concern. BMDL: Benchmark dose level, MOE: Margin of exposure, BW: Body weight.

body weight and high consumption of food products rich in carbohydrates [16]. In addition, children usually consume 35–47% of their daily total caloric intake during the school day [17]. It is, therefore, critically important to estimate the levels of acrylamide in different carbohydrate-rich foods sold in school canteens and to assess the dietary risks that may result from acrylamide consumption during the school days.

Our results for AA levels agreed which was similar to the study conducted in Alexandria city where the levels of AA levels followed the same trend the highest AA levels in potato chips followed by French fries (1500 μgkg^{-1} and 540 μgkg^{-1}, respectively) and the lowest AA level was found in cakes (12 μgkg^{-1}) [18]. There are many factors that may lead to differences in AA level in similar products including the difference in the weight of some ingredients between products, backing temperature, and difference in the added food additives [19].

A remarkable variation in the levels of AA in potato-based products was observed between the different studies. The levels of AA in items made from potatoes varied noticeably between the several experiments. Agamy found that potato products from Egyptian markets vary in AA concentration from 247 to 1677 gkg^{-1} [20]. While the range in comparable items in the Addis Abba study ranged from 36 to 1411 gkg^{-1} for French fries and between 211 and 3515 gkg-1 for potato chips chips [21]. The variation of AA content in fried potato-based products can be attributed to a lot of factors such as free amino acids, reducing sugars concentrations, storage time of potatoes, type of potato cultivar, and the degree of saturation of frying oil [22], [23], [24].

In France, the average dietary exposure of AA among children aging from 7 to 10 years and based on the average dietary exposure to AA from all meals for 7 days/week was only 0.57 $\mu gkg^{-1}bw\ d^{-1}$ [25]. In Jeddah city, Saudi Arabia found that the exposure to AA was higher in primary school students when compared to middle and secondary school students. There was a significant decrease in the mean exposure to AA as the age increased from 0.37 to 0.65 $\mu gkg^{-1}BWsd^{-1}$ [2]. This result was lower than that obtained in the present study where the exposure ranged from 1.34 to 1.45 $\mu gkg^{-1}BWsd^{-1}$ with no statistically significant between educational levels. This difference in exposure to AA can be explained by the differences in canteen products in both studies. Contrary to our study, fried products such as potato chips, French fries, and chicken pane were not allowed to be sold in Saudi Arabia schools. In Canada, they revealed the consumption of deep fried French fries among Canadian teenagers contributed with about 50% of AA exposure followed by potato crisps with 10% then oven baked French fries [10]. In another study, the same pattern was revealed with French fries showing the highest contribution percent (26%) followed by potato crisps (16%) to the overall AA exposure in children and adolescents aged from 7 to 18 years old [11]. Results in the present study also revealed that potato chips contributed to the AA exposure more than French fries since French fries available only in two school canteens while potato chips are available in all schools.

These MOE values in the present study were lower than the values detected in Jeddah schools for the same $BMDL_{10}$ values based on the overall estimated mean intake, the MOE values were 356 and 614 for both values, respectively [2].

Conclusions

In our study, the level of AA was detected in 73 samples for 16 different canteen snacks sold in school canteens. Samples were collected randomly from the canteens of primary schools in Alexandria city. The results showed that the highest level of AA was detected in potato chips (1889 $\mu gkg^{-1)}$ followed by French fries (1757 μgkg^{-1}), while the lowest level for AA was detected in strawberry cake (291 μgkg^{-1}) followed by vanilla cake (298 μgkg^{-1}). Mean dietary exposure to AA among primary school students during school day from canteen snacks was 1.4 μgkg^{-1} $BWsd^{-1,}$ and the exposure level at 95th percentile (higher exposure) was 4.00 μgkg^{-1}. The exposure to AA from consuming canteen snacks among primary school students is considered based on the previously mentioned which requires specific measures by the relevant ministries and authorities to reduce the exposure to AA among primary school students.

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