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Fractional Urinary Fluoride Excretion (FUFE) of 3-4 year children in the Gaza Strip

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A positive association between dental fluorosis prevalence and fluoride (F) concentration in drinking waters has been detected in Gaza Strip. Total Daily Fluoride Retention (TDFR), and Fractional Urinary Fluoride Excretion (FUFE) indicate F body burden; important in assessing fluorosis risk in susceptible age groups. ***Objective:*** 1, To determine and compare Daily Urinary Fluoride Excretion (DUFE) and FUFE of 3-4-year-olds living in lower (<0.7), moderate (0.7-1.2) or higher (>1.2) ppm F tap water areas; 2, To determine any relationship between i, DUFE and tap water F; ii, DUFE and Total Daily Fluoride Intake (TDFI); iii, TDFI and TDFR. ***Methods:*** 24-hour urine and tap water samples were collected from 216 children exposed to lower (n=81), moderate (n=72), or higher (n=63) tap water F. ANOVA with Tukey's Test and Pearson's correlation were used to examine differences in mean DUFE and FUFE and relationships between variables. ***Results:*** Mean drinking water F was 0.11(sd 0.17), 0.14 (sd 0.28) and 0.38 (sd 0.63) ppmF respectively. Differences ($p<0.0001$) in mean DUFEs (0.17 (sd 0.13), 0.25 (sd 0.15) and 0.38 (sd 0.23) mg/day respectively) and mean FUFEs (48 (sd 39)%, 47 (sd 31)% and 63(sd 76)%) were found ($p<0.05$). Significant ($p<0.0001$) positive correlations were found between DUFE and tap water F; DUFE and TDFI, and; TDFI and TDFR. ***Conclusion:*** DUFEs of children drinking waters with 0.11 and 0.14ppm F, represented low F usage. The group drinking 0.38ppmF water represented optimal F usage. The weak significant positive association of DUFE with home tap water F suggests low validity for tap water F in estimating F exposure.

Key words: *urine, metabolism, ingestion, fluorides, child preschool, groundwater, water intake, Gaza Strip, Palestine , FFUE, DFUE, TDFR*

Introduction

A number of studies have identified dental fluorosis as a significant dental health problem among children in the Gaza Strip (Sansur, 1991; Shomar *et al*., 2004). The prevalence of dental fluorosis in permanent teeth has been found to be as high as 78% (Abuhaloob and Abed, 2013). Determining the source of the chronic excessive exposure to fluoride (F) and monitoring the effect of strategies to try and reduce the risk of dental fluorosis in these communities is an important part of oral health improvement in the region. Although the Gaza Strip may not be representative of broader populations, its dental health challenges provide useful information for other communities which may experience similar risks from excessive exposure to F.

Groundwater is the only source of water in the Gaza Strip (Abuzahrah, 1995; Ministry of Health, 2003; Shomar *et al.,* 2004), and a high positive association has been found between the F concentration of municipal ground water supplies for human consumption and the prevalence and severity of dental fluorosis in children (Shomar *et al.,* 2004). However, recent moves towards domestic use of purchased filtered waters and reverse osmosis filters may have changed the relationship between waters used in this environment and dental fluorosis prevalence and severity. In addition, a recent study of 3-4 year olds has shown that drinking waters are not the sole source of F exposure in the Gaza Strip (Abuhaloob *et al*., 2014), other sources being foods, particularly those prepared or cooked with tap water.

In general, ingested fluorides are derived from many sources including water, diet, systemic F supplements and from inadvertent ingestion of toothpastes in young children (Levy, 2003). Historically, several studies have suggested that fluoridated drinking water is the primary source of F intake among children (Fejerskov *et al.,* 1988; Fomon and Ekstrand, 1996; Grimaldo *et al.,* 1995; Harrison, 2005; Levy, 1994), while more recently, no statistically significant correlation was found between the F concentration of home tap waters and total F intake among children in North East England (Maguire *et al.,* 2007; Zohouri *et al.,* 2006a). In addition, in communities using toothpastes routinely, toothpaste ingestion has been found to account for between 35-57% of total daily F intake in 6-7 year-olds (Maguire *et al.,* 2007; Zohoori *et al.,* 2012), although this is not a prime source of F intake in Gaza Strip children since <5% brush their teeth (Abuhaloob *et al*., 2014).

Young children, particularly those in the first four years of life are potentially at risk of dental fluorosis affecting the aesthetically important permanent anterior teeth if they are chronically systemically exposed to excessive F (Dean, 1936; Fejerskov *et al.,* 1988; Hong *et al*., 2006; Levy *et al.,* 2010; TenCate, 1985). To minimize the dental fluorosis risk, it has been suggested that the Total Daily Fluoride Intake (TDFI) of children up to 8 years of age should not exceed 0.05 – 0.07 mg/kg

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body weight/day and, in 4 year old children, it should be within 0.05 – 0.06 mg/kg body weight/day (Burt, 1992), with a tolerable upper intake level (UL) for exposure to avoid dental fluorosis being 0.1 mg/kg bw/day (Institute of Medicine, 1999).

The main route of F elimination from the body is urine (Fejerskov *et al.,* 1988) and therefore urinary F has been proposed as a suitable biomarker for F exposure (Marthaler, 1999; Ruiz-Payan, 2005; Whitford, 2005; World Health Organization, 1984) and for monitoring fluoride intake at a community level (Ketley and Lennon, 2000; Murray, 1986). Other biomarkers for F exposure have been proposed including the measurement of fluoride in toe/fingernails to assess risk for dental fluorosis among children (Buzalaf *et al.,* 2012). However the technical aspects of the collection and F analysis of nail remain challenging and until a simple standardised method demonstrating acceptable validity gains wide acceptance for use at a community level, urinary fluoride excretion remains the preferred biomarker for estimating recent F exposure and fluoride balance (intake minus excretion).

Investigations which have collected Total Daily Fluoride Intake (TDFI) and Daily Urinary Fluoride Excretion (DUFE) data have shown that daily Fractional Urinary Fluoride Excretion (FUFE, the proportion of ingested F which is excreted) varies according to a number of factors including age, rate of growth, diet, and altitude. FUFE has been shown to differ between populations of similar age groups (3-6 years old); from approximately 80% in Iran (Zohouri and Rugg-Gunn, 2000), to 51.5% in Germany (Haftenberger *et al.,* 2001), and 30.7 % in Chile (Villa *et al.,* 1999), while in the UK, in 3-5 year olds it has been found to be approximately 30% (Murray, 1986; Ketley *et al.,* 2002; Villa *et al.,* 1999). This means that, on average, around 55% of TDFI is retained in a child's body (Villa *et al*., 2010), but the differences in FUFE seen between populations raises the importance of its estimation for each population, especially in areas with substantial exposure to F from different sources in which individuals may be at risk of skeletal or dental fluorosis.

Despite widespread use of urinary F excretion measurement as a valid and non-invasive indicator for TDFI, there is no information on DUFE in the Gaza Strip, nor any information about to what extent F in the home tap water supply might contribute to DUFE. This study was part of a larger project in three areas of Gaza Strip with different F concentrations in tap waters supplied and drinking and cooking waters consumed. The mean (sd) F concentrations of drinking waters in the areas designated as lower, moderate and higher F areas were 0.11 (0.17), 0.14 (0.28) and 0.38 (0.63) ppmF respectively, values which were lower than the mean F in tap waters (0.21 (0.15), 0.83 (0.28) and 1.52 (0.52) ppmF respectively which were used for cooking in 87.7%, 84.7% and 84.1% of the households respectively). The mean (sd) TDFIs were 0.02(0.01), 0.04(0.01) and 0.05(0.03) mg/kg body-weight/day respectively and foods made the largest contribution (63.9%) to TDFI with toothpaste accounting for only 14% of TDFI in the <5% of children who used a toothbrush (Abuhaloob *et al.,* 2014).

The aim of this currently reported aspect of the wider project was to determine the extent to which the F concentration of supplied home tap water impacted on the 24 hour urinary F excretion of the same 3-4 year olds living in the Gaza Strip, by examining the relationship between their measured DUFE and F concentrations in supplied home tap water and consumed drinking water. The relationship between the DUFE and measured TDFI as well as FUFE and Total Daily Fluoride Retention (TDFR) was also estimated and compared between children living in areas receiving different tap water F concentrations. This information was compared with the World Health Organization's (WHO) guidelines (Marthaler, 1999), which provide provisional standards for DUFE related to F usage. These guidelines are produced to aid reliable monitoring of F exposure in communities and facilitate the optimisation of the benefits of fluorides while reducing dental fluorosis risk. A subsidiary aim of this study was to provide baseline F exposure data and information for future monitoring studies in the Gaza Strip as well as in other low income countries with concerns about water quality in relation to F exposure.

Materials and Methods

Ethical aspects were considered and relevant approvals secured including those of the Palestinian Ministry of Health, the United Nations Relief and Works Agency for Palestine Refugees in the Near East (UNRWA) and the local municipal authority.

Groundwater is the main source of water supply in the Gaza Strip (Shomar *et al*., 2004; Palestinian Central Bureau of Statistics, 2007). Municipally supplied groundwater is provided in Gaza although additionally some of the population have their own private wells and may use other purchased or filtered waters for drinking and cooking (Abuhaloob *et al*., 2014). A preliminary fluoride analysis of morning and evening tap water samples collected over three consecutive days from houses covering all governorates of Gaza Strip was undertaken. Based on the F concentration of these samples, three study areas for subject recruitment were selected: 1, Beit Lahia and Alnaser; areas of lower F (<0.3ppm) in tap water; 2, Albureej; an area of moderate F (0.7–1.2 ppm) in tap water; 3, Alshaaf; an area of higher F (>2ppm) in tap water.

All health records of children aged 3-4 years of age in the selected areas were identified within governmental and UNRWA health care centres and a random selection of 100 healthy children in each area listed. The parents of the first 72 children on each list were contacted by telephone to explain the study and determine any expression of interest in the study. Those parents expressing interest were visited by the researcher who provided an explanatory letter about the study, a parental information sheet, and answered any questions the parents had regarding the study, before gaining informed written consent. If any parent declined to consent or their child did not meet the study criteria, the parents of the next child on the list was contacted until 72 children in each area had been recruited.

The study inclusion criteria were having lived in the same area since birth, not receiving any dietary F supplements or professionally applied F and not reporting persistent bed-wetting. The exclusion criteria were: child not aged 3-4y at the time of data collection; having any kind of health problem including chronic metabolic and

renal diseases; receiving any F supplement or F therapy; not being continuously resident in the selected area since birth or planning to move from the area in the following year; or who reported consistent bed-wetting when questioned. The study was carried out during the summer season when mean daily temperatures average 25°C and the humidity can increase from 65% in the morning to more than 80% in the evening (Shomar *et al*., 2004).

Following consent, parents received a further detailed explanatory letter about the study and the 24 hour urine collection procedure. The parents were given a F-free disposable jug, disposable funnel and disposable plastic bottle (2 litres) and were asked to collect and record the time and volume of all urine passed by their children from the second passing of urine of the first day, through to the first urine passed the next morning, in order to provide a complete 24 hour urine collection. At an interview on the second day parents were asked about the completeness of the urine sample and any parents who had forgotten to collect any urine voiding of their children were asked to collect another 24 hour urine sample the following day (Zohouri and Rugg-Gunn, 2000).

The volume of each 24h urine sample was measured and 5 ml aliquots were stored in a freezer at -20°C prior to transportation to the UK.

For each child, their height and weight was measured; vertical height without shoes using a digital stadiometer (Soehnle, Germany) to the nearest 0.5 cm, and weight using digital scales (Soehnle, Germany) without shoes and outdoor clothing to the nearest 0.5 kg. The same scales and stadiometer were used throughout the study.

Parents collected home tap water samples of similar volumes (25ml) for three consecutive days. These were mixed and 5ml aliquots stored in a freezer at -20°C prior to transportation to the UK.

Once the necessary local permissions and UK Department of the Environment and Rural Affairs (DEFRA) licences were secured, all collected urine and tap water samples were transported to the UK frozen on dry ice at -20°C and kept frozen at -20°C until F analysis.

To determine the completeness of each urine sample collection, its creatinine concentration was analysed using the Jaffé reaction (Bonsnes and Taussky, 1945) and 24h urinary creatinine excretion calculated. Those samples which met one or both of the two validation criteria; a urine flow rate >140ml in 24h and a urinary creatinine concentration between 0.1 and 1.5 mg/ml, were included in the data analysis (Marthaler, 1999). The creatinine based method for validation of urinary excretion is described in detail elsewhere (Zohouri *et al*., 2006b)

Urine and tap water samples were analysed in triplicate at room temperature using a direct method (Martinez-Mier *et al.,* 2011; Venkateswarlu and Vogel, 1996) using Total Ionic Strength Adjustment Buffer (TISAB III) and a fluoride ion-selective electrode (Model 720 A+: Thermo Orion, US), calibrated against a standard curve based on millivolt readings for fluoride standards.

The Daily Urinary Fluoride Excretion (DUFE), recorded in mg and in mg/kg bw/day for each child, was determined from the F concentration (mg/Litre) and volume of the 24 hour sample (Litres), corrected for 24h by recording the actual time period over which the sample was collected, starting after the first void on day 1 (start time) and including the first void of day 2 (stop time), in line with WHO guidelines (Marthaler, 1999) Total Daily Fluoride Retention (TDFR), recorded in mg, was calculated assuming a constant value of 90% for F absorption as a proportion of Total Daily Fluoride Intake (Villa *et al*., 2010); TDFR = (0.9 x TDFI) – DUFE.

The FUFE (%) was calculated as (DUFE/TDFI)×100, where TDFI was estimated using a three-day diary to record the amount and frequency of food and drink items consumed, along with F analysis of the associated consumed food and drink items. The method used to estimate TDFI in this study is described fully elsewhere (Abuhaloob *et al*., 2014).

Data were entered into SPSS v.20 software for analyses. Descriptive analyses were presented using mean (sd) of the variables. One way ANOVA and a Tukey Post Hoc test were used to examine the statistical significance of the differences in the mean DUFE and FUFE of children exposed to lower, moderate and higher F in home tap water supplies. The relationship between: i, DUFE and F concentration of home tap water; ii, DUFE and F concentration of actual drinking water; iii, DUFE and TDFI; and iv, TDFI and TDFR were all determined with Pearson's correlations. The statistical analysis was implemented at $p<0.05$ with 95% confidence intervals.

Results

Overall, 216 children (112 females and 104 males) aged 42 to 56 months, mean 4.06yr (sd 0.32) were recruited from the 225 contacted, 96% participation. Most children's mothers were housewives and unemployed (94%). Of the participants' fathers: 26% were unemployed, 28% were in the service industries including shop and market workers; 24% were employed in craft and related trades. Most of the families (87%) lived under the poverty line and had monthly incomes ≤1800 Israel New Shekels (Palestinian Central Bureau of Statistics, 2004), which is currently equivalent to ≤525US$.

Table 1 shows that the weights and heights of children were similar across the three areas of F concentrations in home tap water.

All 216 urine samples met the urinary creatinine concentration criterion for validation of completeness while 200 met the flow rate criterion and therefore all 216 samples were included in the data analysis. The mean urinary creatinine concentration was 0.37mg/ml (sd 0.18, range 0.10-1.10). The mean urine volume was 303ml (sd 150). The Coefficient of Variation for F

Table 1. Mean (sd) weight and height of 3-4yr-olds receiving tap water of low, medium and high F concentrations

	Fluoride concentration in tap water					
	Low, 0.21ppm n=81		*Medium, 0.91ppm n=72*		*High, 1.71ppm n=63*	
	mean	*(sd)*	*mean*	*(sd)*	*mean*	*(sd)*
Height, cm	100.2	(4.3)	101.4	(4.5)	100.7	(4.6)
Weight, kg	16.2	(1.9)	16.2	(2.2)	16.2	(2.2)

Table 2. Fluoride concentration in supplied tap waters, F concentration in actual drinking waters, 24h urinary F concentration, and Daily Urinary Fluoride Excretion (DUFE) of 216 3-4 year-olds (n=216)

F areas based on tap water F (ppm)	*n*	*F concentration in tap water samples (ppmF)*		*F concentration in drinking water samples (ppmF)*		*24 h urinary F concentration (ppmF)*		*DUFE (mg)*		*DUFE (mg/kg bw)*	
		mean	*(sd)*	*mean*	*(sd)*	*mean*	*(sd)*	*mean*	*(sd)*	*mean*	*(sd)*
Lower	81	0.21	(0.15)	0.11	(0.17)	0.62	(0.42)	0.17	(0.13)[1]	0.011	(0.008)
Moderate	72	0.91	(0.13)	0.14	(0.28)	0.93	(0.48)	0.25	(0.15)[1]	0.016	(0.009)
Higher	63	1.71	(0.35)	0.38	(0.63)	1.28	(0.69)	0.38	(0.23)[1]	0.023	(0.013)

[1] One Way ANOVA: p<0.0001. Comparing DUFE values between the three F concentration areas revealed significant differences in all three cases with p values of <0.01 when comparing moderate v low areas and <0.001 in the other cases (Tukey's post-hoc test)

Table 3. Fluoride concentrations in tap and drinking waters, measured Total Daily Fluoride Intake (TDFI), Total Daily Fluoride Retention (TDFR) and Fractional Urinary Fluoride Excretion (FUFE, %) of 216 3-4 year-olds.

Fluoride concentration in home tap water (ppm)		*Fluoride concentration in drinking water (ppm)*		*Number of children*	*Measured TDFI (mg/day)*		*Measured TDFI (mg/kg bw/day)*		*TDFR (mg)*		*TDFR (mg/kg bw/day)*		*FUFE (%)*	
mean	*(sd)*	*mean*	*(sd)*	*n*	*mean*	*(sd)*	*mean*	*(sd)*	*mean*	*(sd)*	*mean*	*(sd)*	*mean*	*(sd)*
0.21	(0.15)	0.11(	0.17)	81	0.389	(0.153)	0.02	(0.01)	0.181	(0.172)	0.011	(0.011)	47.7	(39.4)
0.91	(0.13)	0.14	(0.28)	72	0.572	(0.214)	0.04	(0.01)	0.262	(0.120)	0.017	(0.013)	47.3	(31.0)
1.71	(0.35)	0.38	(0.63)	63	0.791	(0.446)	0.05	(0.03)	0.335[1]	(0.468)	0.021[1]	(0.027)	63.4	(76.0)
Overall				216	0.567	(0.329)	0.04	(0.02)	0.253	(0.302)	0.016	(0.018)	52.2	(51.1)

[1]Statistically significant at p<0.05 Comparing TDFR values (in mg/day and mg/kg bw/day) between the three F concentration areas revealed a statistically significant difference only in the case of high v low concentrations with p values of 0.006 and 0.0003 respectively (one way ANOVA and Tukey's post-hoc test).

analysis of water samples was ≤2.81% indicating very good reproducibility, while the mean recovery of F in urine samples was 99.3% and so within an acceptable range of 98% to 103%.

Table 2 describes the mean F concentrations of the supplied tap waters and actual drinking waters for the three groups of children and DUFE. Statistically significant differences in DUFE were found between children supplied with the lower, moderate and higher F home tap waters. A significant (r=0.42, p<0.0001, R^2=17.8%) moderate positive correlation was observed between DUFE and F concentration in tap water; the tap water F concentration accounted for approximately 18% of the variance in DUFE (Figure 1).

The TDFI, TDFR and FUFE by group are presented in Table 3. The mean (sd) TDFI increased with increasing F concentrations in the supplied and consumed waters from 0.02 (0.01) mg/kg bw/day in the lower F area (tap water F=0.21 (0.15) ppm; drinking water F=0.11 (0.17) ppm) to 0.05 (0.03) mg/kg bw/day in the higher F area (tap water F=1.71(0.35) ppm; drinking water F=0.38 (0.63) ppm). The TDFR ranged from 0.181 (0.172) mg in the lower F area to 0.335 (0.468) mg in the higher F area, a difference which was highly statistically significant (p<0.006). This level of body F retention was also represented by the mean overall FUFE of 52 (sd 51)% which when expressed according to area showed FUFE to be similar in the lower and moderate F areas at 48

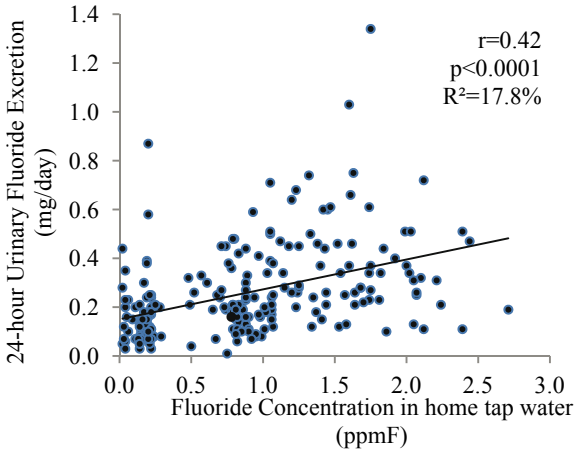

Figure 1. *Relationship between Daily Urinary Fluoride Excretion (DUFE) (mg/day) and F concentration in home tap water (ppmF) in 216 children aged 3-4y*

(sd 39)% and 47 (sd 31)% and higher at 63 (76)% in the higher F area. When the relationship between measured TDFI and DUFE was considered, a weak statistically significant positive correlation was found (Figure 2).

The relationship between TDFI and TDFR is described in Figure 3 and shows a strong positive statistically significant correlation between these two variables with TDFI accounting for 64.2% of the variance in the F retention.

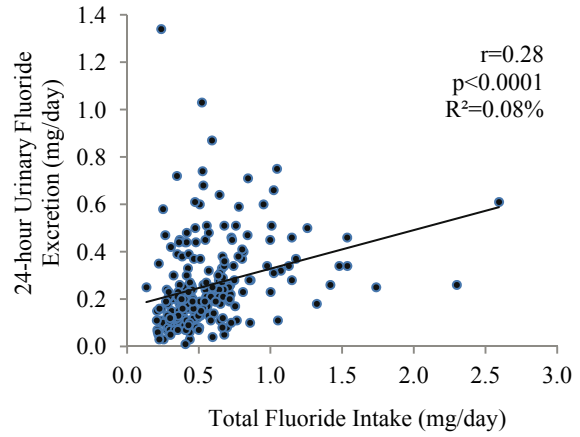

Figure 2. *Relationship between Total Daily Fluoride Intake (TDFI) in mg/day and Daily Urinary Fluoride Excretion (DUFE) in mg/day for 216 children aged 3-4y*

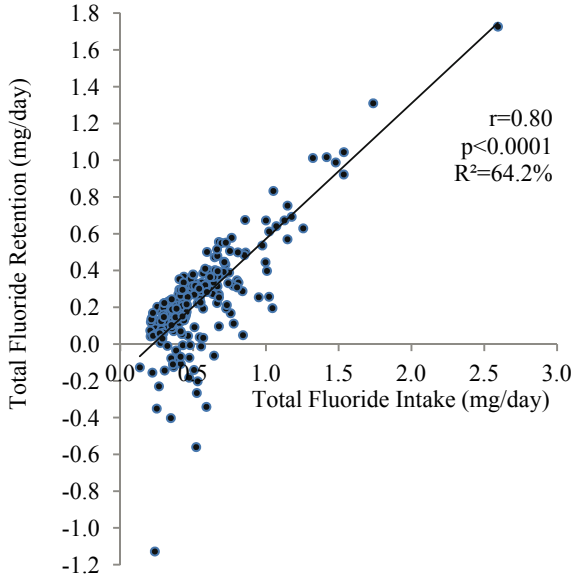

Figure 3. *Relationship between Total Daily Fluoride (TDFI) (mg/day) and Total Daily Fluoride Retention (TDFR) (mg/day) for 216 children aged 3-4y*

Discussion

Collecting 24 hour urine samples in young children is challenging and a number of alternative collection methods have been proposed for measuring urinary fluoride excretion, including using the F/Creatinine ratio in a morning spot urine sample (Kertesz *et al*., 1989; Zohouri *et al*., 2006b). In view of the limited literature on these other methods and the current recommendations of the WHO, this study preferred to use the 24h urinary collection, using WHO validation criteria to determine the final dataset for analysis. This method is made more robust when multiple 24 hour urine collections are possible, but that was beyond the practicalities of this study, in which urine collection was synchronised with dietary data collection. However, in terms of the feasibility of collecting 24 hour urine, by four years of age a child is usually in control of their urine excretion (Rugg-Gunn *et al.,* 1993).The WHO advises that the 24 hour urinary creatinine concentration for a complete 24h urine sample should lie between 0.1 and 1.5 mg/ml for all ages. Using this value as a validating criterion (Marthaler, 1999), the 24 hour urinary creatinine concentration for all 216 participants in the current study was between 0.1 and 0.9 mg/ml and therefore within the limits of the WHO cleaning criteria for determining completeness of the 24 hour urine sample.

In addition, the WHO (Marthaler, 1999) recommends the discarding of urine samples with volumes less than 140 ml/24 hour or more than 1200 ml/24 hour as criteria for collection of 24 hour urine samples in children <6 years of age. Several previous studies have discarded 24 hour urine samples when 24 hour urinary flow rates were lower or higher than these recommended limits, assuming that these samples are either incomplete or had been diluted with water and thus did not represent an accurate record of 24 hour urine production (Franco *et al.,* 2005; Ketley *et al.,* 2004; Ketley and Lennon, 2000; Marthaler *et al.,* 1995). Currently no one validating criterion for determining completeness of urine collection has been found to be more useful than another (Marthaler, 1999) as they can differ according to diet, activity and climate. Until there is evidence suggesting the use of one criterion in preference to another, in terms of accuracy and reliability, then the decision to include urine samples meeting at least one criterion for completeness would seem to be justified.

In the present study the mean DUFEs of 0.17 and 0.25 mg/day of children supplied with tap waters with a mean F concentration of 0.21ppm and 0.91ppm respectively, lay within the WHO's provisional standards for DUFE for children exposed to "low F intake"; 0.17- 0.29 mg F for 3-5 year olds. Furthermore, the mean 24 hour urinary F excretion in children who received tap water with a mean F concentration of 1.71ppmF in the present study (0.38 mg) was within the 0.36 – 0.48 mg F range reported by the World Health Organization as representing conditions of "optimal fluoride usage" (Marthaler, 1999). This latter result was slightly surprising initially, but was most likely due to the mean F concentration for the actual drinking water consumed for this group being 0.38ppmF. In addition, the WHO guidance is based on a small number of studies carried out in different populations and therefore it is important that they be considered in this light. The recommended values for DUFE are also not currently described on a per kg body weight basis which would be more appropriate in view of the body metabolism they represent.

As Table 4 shows, urinary fluoride excretion studies undertaken with similarly aged children in some lower F (<0.3ppmF) European areas and Jamaica show the mean (sd) DUFE of children to be close to that of the studied children in the Gaza Strip (0.17 (0.13)) who were receiving a mean tap water F concentration of 0.21 (0.15) ppmF, but were actually drinking water with a mean F concentration of 0.106 (0.170) ppmF. However, in other lower F areas of England, the Netherlands, Portugal and Iran the higher urinary F excretions found contrast with those found for children receiving similar F concentra-

Table 4. Summary of studies of Daily Urinary Fluoride Excretion (DUFE) in similarly aged children (3-4y) living in low fluoride areas

Study authors (publication date)	*Location of study*	*Age (years)*	*No. of children*	*Water F (ppmF)*	*Mean (sd) Daily Urinary Fluoride Excretion (mg/d)*
Warpeha and Marthaler (1995)	Kingston, Jamaica	2 - 6	7	<0.25	0.17 (0.08)
Zohoori and Rugg-Gunn (2000)	Fars region, Iran	4	78	0.30 - 0.39	0.34 (0.10)
Ketley *et al.* (2004)	Reykjavik, Iceland	2.3 - 3.9	4	<0.15	0.10 (0.01)
	Oulu, Finland	2.3 - 3.6	18	<0.15	0.16 (0.08)
	Knowsley, England	1.8 - 4.2	18	<0.15	0.20 (0.14)
	Haarlem, Netherlands	2.6 - 4.0	6	<0.15	0.21 (0.15)
	Almada/Sebutal, Portugal	2.5 - 3.6	21	<0.15	0.33 (0.27)
Present study (2014)	Gaza Strip, Palestine	3.5 - 4.5	216	mean 0.21 (sd 0.15) tap water mean 0.11 (sd 0.17) drinking water	0.17 (0.13)

tions in tap water in the Gaza Strip. Since the Gaza Strip children were drinking water with a lower mean F concentration, it is therefore most likely that this, as well as differences in F intake from other sources such as from drinks or foods prepared with fluoridated water or ingestion of fluoridated toothpaste, may account for some of the differences seen.

It has been shown that the mean DUFE of 1.5 to 3.5 year-olds living in fluoridated (0.8-1.0ppmF) areas of Europe was statistically significantly higher than that in lower F (<0.15ppmF) areas ($p=0.003$) (Ketley *et al.,* 2004); a result similar to that found in this study ($p<0.01$).

In contrast, 2.5 - 4 year-olds who consumed water with a F concentration ranging between 0.7 and 1.2ppm in Cork (Ireland), Dambulla (Sri Lanka) and Newcastle upon Tyne (UK) had DUFEs of approximately 0.37mg (Ketley *et al.,* 2004), 0.55mg and 0.42mg (Rugg-Gunn *et al.,* 1993) respectively; higher than the urinary F excretion of children in the present study which was 0.25mg in those receiving a mean tap water F concentration of 0.91ppmF and consuming drinking water with a mean F concentration of 0.14ppm.

In general, the current study illustrated that the DUFE of children in the Gaza Strip was lower compared with previously published data from other countries, and this may be attributed to the increasing trend towards consumption of purchased drinking water (e.g. filtered and lower in fluoride as described in Table 2) seen in these communities, in combination with the supplied tap water used less for drinking and more for cooking (Abuhaloob *et al.,* 2014),. The weak correlation of DUFE v F concentration of tap water illustrates this relationship and highlights one reason why studies may have shown differences in the validity of home tap water F for predicting F exposure.

The information on TDFI collected in the wider aspects of this study (Abuhaloob *et al*., 2014) provided the opportunity to investigate the relationship between estimates of TDFI intake and DUFE and this showed a weak positive statistically significant relationship between these two variables.

Work by Villa *et al*., (2010) which considered data from the simultaneous measurement of TDFI and DUFE in 212 children aged <7 years derived a regression equation to estimate TDFI from DUFE; TDFI = (DUFE-0.03)/0.35. Based on this equation, in the current study the estimated TDFI based on DUFE would be 0.025 (0.02), 0.039 (0.026) and 0.061 (0.037) mg F/kg bw/day in the three groups. When compared with their measured TDFI which was 0.02 (0.01), 0.04 (0.01) and 0.05 (0.03) mg/kg bw/day respectively, the equation showed good predictive quality for children at lower F exposures, which may reflect a similar range of exposures used in the original dataset used to determine the regression equation. As more F exposure data reflecting different patterns of use and sources of fluorides become available, it would be useful to add these to the dataset and increase its power and accuracy in the estimation TDFI from DUFE. This in turn would provide a powerful tool and further facilitate monitoring of F exposure and F-based community prevention programmes.

Although the correlation between DUFE and F concentration in supplied tap water was statistically significant, it was only moderate (at $r=0.422$) and this suggests that tap water F concentration is not an appropriate indicator for total F exposure, mainly due to the presence of other significant sources of F intake. Similarly, Rugg-Gunn *et al.,* (1993) illustrated that, even though the 2-4y old children in Dambulla, Sri Lanka (average temperature 27°C) and Newcastle upon Tyne (average temperature 12°C) consumed water with a similar F concentration (0.7-1.2ppmF), there was a statistically significantly greater DUFE in the Sri Lankan children, which was attributed to the differences in F intake from diet, diet type and the volume of water intake, due to differences in ambient climate. Children who are exposed to the same F concentration in consumed water will not necessarily have similar DUFE; other factors must be considered including diet and eating habits, F ingestion through toothbrushing, water (and drinks) consumption per day and local as well as seasonal climates.

Burt (1992) suggested that the F intake of children up to 8 years old should not exceed 0.05 – 0.07 mg/kg body weight/day, and recommended that the TDFI for 4 year old children should be within the limits of 0.05 – 0.06 mg/kg body weight/day. Overall, the children in this study did not meet this intake with mean (sd) TDFIs of 0.02 (0.01), 0.04 (0.01) and 0.05 (0.03) mg/kg bw/day respectively in areas where the mean tap water F concentrations were 0.21 (0.15), 0.91 (0.13) and 1.71 (0.35) ppmF but where the mean F concentrations of waters actually used for drinking were 0.11 (0.17), 0.14 (0.28) and 0.38 (0.63) respectively and toothbrushing was rarely practised; only 11 of the 216 children brushed their teeth (Abuhaloob *et al.,* 2014).

Many factors can influence F intake, retention and excretion both within and between populations but it is ultimately F retention which impacts F body burden and the potential risk of fluorosis. In this study the mean (sd) FUFE was 52.2 (51.1)% but ranged from 47.7 (39.4)% in the 0.91ppmF tap water area to 63.4 (76.0)% in the 1.71ppmF tap water area and showed a wide variance. This is very similar to the 55% F retention rate described for 212 children aged <7 years by Villa *et al*. (2010).

A weak statistically significant positive relationship was found between DUFE and F concentration in supplied tap water but the tap water F concentration accounted for only 18% of the variance in DUFE suggesting that it should not be used as an index of F exposure.

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