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Evaluation of Trace Elements in Malay Women with Type 2 Diabetes Mellitus

(Penilaian terhadap Unsur Surih dalam Kalangan Wanita Melayu dengan Diabetes Melitus Jenis 2)

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ABSTRACT

Trace elements are essential for human health. This study determines the level of chromium (Cr), zinc (Zn), selenium (Se), copper (Cu), ferrum (Fe) and manganese (Mn) in the hair and nails of married Malay women with type 2 diabetes mellitus from Hulu Langat, Selangor, Malaysia. The trace elements were analysed using inductively coupled plasma mass spectrometry (ICP-MS) after an acid digestion treatment. The levels of all trace elements measured were higher in the hair samples than in nails except for Se. Mean levels of Zn and Se were significantly higher in the hair of non-working women (84.91 ± 10.32 µg/g and 0.42 ± 0.15 µg/g, respectively) compared to working women (64.98 ± 11.10 µg/g and 0.30 ± 0.18 µg/g). Copper levels in hair were significantly higher among women with good and satisfactory diabetes control (20.41 ± 4.20 µg/g) compared to the poor diabetes control group (15.67 ± 4.31 µg/g). However, a contradictory result was obtained from the nails of women with good and satisfactory diabetes control with a Cu level of 3.35 ± 0.46 µg/g and 4.69 ± 0.33 µg/g for the poor diabetes control group. In hair samples, significant positive correlations were obtained with Cr-Cu (r = 0.55), Zn-Mn (r = 0.46) and Se-Mn (r = 0.38). In nails, significant positive correlations were also obtained between Cr-Zn (r = 0.31), Zn-Fe (r = 0.30) and Fe-Mn (r = 0.47). In conclusion, age, educational status, number of children and duration of diabetes did not influence trace elements levels.

Keywords: Diabetes mellitus; female; HbA1c; ICP-MS; trace elements

ABSTRAK

Unsur surih adalah penting untuk kesihatan manusia. Kajian ini akan menentukan tahap kromium, zink (Zn), selenium (Se), kuprum (Cu), ferum (Fe) dan mangan (Mn) dalam rambut dan kuku wanita Melayu yang sudah berkahwin dengan diabetes melitus jenis 2 dari Hulu Langat, Selangor, Malaysia. Unsur surih telah dianalisis dengan menggunakan spektrometri jisim plasma gandingan aruhan (ICP-MS) selepas rawatan asid penghadaman. Tahap semua unsur surih yang diukur adalah lebih tinggi dalam sampel rambut daripada kuku kecuali Se. Tahap min Zn dan Se adalah jauh lebih tinggi dalam rambut wanita tidak bekerja (masing-masing 84.91 ± 10.32 µg/g dan 0.42 ± 0.15 µg/g,) berbanding wanita bekerja (64.98 ± 11.10 µg/g dan 0.30 ± 0.18 µg/g). Tahap kuprum dalam rambut adalah jauh lebih tinggi dalam kalangan wanita dengan kawalan diabetes yang baik dan memuaskan (20.41 ± 4.20 µg/g) berbanding dengan kumpulan kawalan diabetes melitus yang tidak dikawal (15.67 ± 4.31 µg/g). Walau bagaimanapun, keputusan bercanggah telah diperoleh daripada kuku wanita dengan kawalan diabetes yang baik dan memuaskan dengan tahap Cu ialah 3.35 ± 0.46 µg/g dan 4.69 ± 0.33 µg/g bagi kumpulan kawalan diabetes melitus yang tidak dikawal. Pada sampel rambut, korelasi positif yang signifikan diperoleh dengan Cr-Cu (r = 0.55), Zn-Mn (r = 0.46) dan Se-Mn (r = 0.38). Pada kuku, korelasi positif yang signifikan juga diperoleh antara Cr-Zn (r = 0.31), Zn-Fe (r = 0,30) dan Fe-Mn (r = 0.47). Kesimpulannya, umur, tahap pendidikan, bilangan anak dan tempoh diabetes tidak mempengaruhi tahap unsur surih.

Kata kunci: Diabetes melitus; HbA1c; ICP-MS; perempuan; unsur surih

INTRODUCTION

Diabetes mellitus (DM) is a disorder of multiple etiologies characterised by chronic hyperglycemia with disturbances of the carbohydrate, fat and protein metabolism resulting from defects in insulin secretion (type 1 DM) or action (type 2 DM). The prevalence of diabetes is increasing globally with the most rapid increases occurring in developing countries including the Asian region and among younger age groups (Rhee 2015). These increasing prevalence rates have been found to be associated with urbanisation which is associated with sedentary lifestyles, ageing and unhealthy diets. According to the World Health Organisation, the number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014 (WHO 2016). The Fifth National Health and Morbidity Survey (2015), showed that the overall prevalence of DM among Malaysians was 17.5%. DM was found to be associated with increasing age, positive family history of DM, obesity, and lower levels of education (Fifth National Health and Morbidity Survey 2015; Rampal et al. 2010).

Interest in trace elements has been increasing steadily over the last few decades. Trace elements are essential for optimum human health due to their diverse metabolic characteristics and functions. Furthermore, these elements are involved in glucose homeostasis and metabolism. Moreover, previous studies have shown that the metabolism of several trace elements is altered in DM, suggesting specific roles for trace elements in the pathogenesis and progress of this disease (Flores et al. 2011; Khalid et al. 2014). At a cellular level, chromium maintains insulin receptors, enhances insulin sensitivity and improves insulin action in moving glucose into the cells (Unjiati et al. 2015). A recent report found that newly diagnosed diabetes patients have chromium levels lower than non-diabetes individuals (Unjiati et al. 2015).

Preclinical investigations showed that zinc supplementation has insulin-enhancing effects, improves β-cell function and lowers blood glucose (Wolfgang 2017). The antioxidant functions of selenium are attracting more researchers due to its important association with DM (De Vega et al. 2016). Selenium deficiency was observed in the association with many illnesses including some types of cancer, heart disease and DM. (Rayman 2000). A meta-analysis has reported that levels of copper in DM patients are higher than healthy people (Qiu et al. 2017).

Additionally, an imbalance of copper and other transition metals contribute to the generation of advanced glycation end products (AGES) and reactive oxygen species (ROS) (Lowe et al. 2017). Previous studies reported that high levels of iron (Fe) contributed to the incidence of type 2 DM and associated with insulin resistance (Altamura et al. 2017). Also, Mn supplementation improved manganese superoxide dismutase (MnSOD) activity and showed protection against complication of type 2 DM (Burlet & Jain 2013; Lee et al. 2013).

In Malaysia, little is known about the status of trace elements among diabetic patients. Thus, this study evaluates trace elements among Malay women with type 2 DM. Six essential elements were studied: chromium, zinc, copper, iron, manganese and selenium from scalp hair and fingernails specimens. The status of these elements was compared between subjects according to their socio-demographic data and status of diabetes control.

Materials and Methods

Study Design and Subjects

A cross-sectional study was carried out among 39 Malay women with type 2 diabetes in the Hulu Langat district of Selangor state, Malaysia (2009-2010). The following criteria were used to select the subjects: Malay women aged between 20 and 60 years and diagnosed with type 2 DM, while exclusion criteria included suffering chronic or terminal illness such as heart disease, cancer and kidney disease, psychiatric illness with ongoing medication, type 1 DM, pregnancy or two months post-partum and having stained hair and/or nails. Informed consent was sought and obtained from subjects before recruitment into the study. Subjects' samples were collected at Universiti Kebangsaan Malaysia.

Questionnaire

A pre-tested questionnaire and face-to-face interviews were used to collect data on the socio-demographic status, health status and the medical history of the subjects. The first section of the questionnaire was related to respondents' demographic data which include name, address and contact, identity card number, date of birth, age and family income. The second section was related to the history of diabetes, health status and the drugs and supplements used by the subjects.

Samples Collection

A sample of nails was collected from each participants using a stainless-steel clipper. About three to five strands of scalp hair (occipital area) were collected and stored in a closed plastic bag at room temperature until the time of analysis. Three mL of blood were collected in ethylenediaminetetraacetic acid (EDTA) tubes and sent to a private laboratory for haemoglobin A1c (HbA1c) estimation.

Sample Washing

Hair and nail samples were washed according to the methods of Batista et al. (2008) and Miekeley et al. (1998). The washing procedures were performed in an ultrasonic bath (model-5510, Branson Ultrasonic Corp, USA). First, the samples were washed with 20 mL of acetone (ultrapure) for 10 min. After three short rinses with water to remove the organic solvent, the samples were washed three times for 10 min each with 20 mL of 1% Triton X-100®. The detergent types and washing times are chosen to ensure the maximum cleaning of external contamination to avoid loss of endogenous elements. The samples were rinsed with Milli-Q water to remove the detergent and then the samples were dried in an oven at 60°C for overnight.

Sample Dissolution and Preparation

Samples digestion was performed according to the method of Miekeley et al. (1998) with little modification due to low sample weight. Hair or nail samples were wet washed with 0.5 mL of HNO3 (p.a. grade) in a closed graduated polypropylene tube (15 mL) overnight at room temperature. Then, the samples were incubated for 1 h at 60-70°C in a drying oven. After cooling, 0.2 mL H2O2 (30% v/v) was added, and the samples were incubated again for 1 h at 70°C. After that, the samples were diluted to a final volume of 10 mL with Milli-Q water 18.2 MΩ-cm.

Preparation of Blank

Blank was prepared in a 25 mL volumetric flask by adding 0.5 mL of 65% HNO3 and 0.2 mL H2O2. Then, the volume was made up to 10 mL with Milli-Q water at 18.2 MΩ-cm. This volume was transferred to a 15 mL closed graduated polypropylene tube.

ICP-MS CALIBRATION AND SAMPLE ANALYSIS

Calibration was performed by preparing six serial concentrations for the multi-element standard solution 2, 8, 20, 50, 100, and 250 µg/L and a calibration graph was plotted. Sample standard and blank were analysed using inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Sciex Elan 9000, USA). All glass and plastic wares such as tubes, volumetric flasks, cylinder and pipettes, tips and containers were cleaned before use according to Talbot and Weiss (1994). These items were soaked with an analytical grade nitric acid solution and ultrapure water overnight and then rinsed three times with Milli-Q water. After that, all the glass and plastic wares were dried in a clean oven and kept in a closed container to prevent contamination. Table 1 lists the equipment and operating conditions used in this study.

STATISTICAL ANALYSIS

TABLE 1. Equipment and operating conditions used in this study

ICP-mass spectrometry	
Instrument	Perkin Elmer Elan 9000
RF-power	1000W
Plasma gas flow rate	20 L/min
Spray chamber	Ryton double pass
Cone	Nickel
Resolution	0.7 ± 0.1 amu
Isotopes measured	Cr-52, Zn-66, Se-82 Cu-63, Fe-57, Mn-55
Dwell time	250 ms
Sweeps	20 per reading
Replicate	Three

The Statistical Package for Social Sciences for Windows (SPSS version 18.0) was used for statistical analysis of the data. The normality of elements levels was examined using the Shapiro-Wilks test and was found to be normal. Pearson's correlation was used to test the correlation between elements, and t-test and/or ANOVA was used to compare the levels of the elements between groups whereby $p< 0.05$ was considered statistically significant.

RESULTS

Thirty-nine Malay women patients aged 25 to 60 years with a mean age of 43.95 ± 8.7 years and diagnosed for type 2 DM who had received treatment at three clinics in the Hulu Langat district of Selangor state, Malaysia, participated in this study. The basic characteristics of the subjects are shown in Table 2. All the subjects were married and about three-quarters of them showed poor diabetes control based on the measurement of glycated haemoglobin (HbA1c). The mean ± S.D of HbA1c for poor control group was significantly higher than good control group at 10.49 ± 1.97 compared to 6.19 ± 0.42 (t= -10.994, $P < 0.05$).

TABLE 2. Basic characteristics of the subjects

Variables	Frequency	Percentage (%)
Age		
20-30	4	10.3
31-40	8	20.5
41-50	19	48.7
51-60	8	20.5
Education status		
Primary school	8	20.5
Secondary school	21	53.8
Diploma/STPM	9	23.1
Employment status		
Working	20	51.3
Not working	18	46.2
Number of children		
<4	21	53.8
≥4	16	41.0
Diabetes control		
Good	10	25.6
Poor	29	74.4

LEVELS OF TRACE ELEMENTS

Tables 3 and 4 present the mean levels of trace elements in the hair and nails specimens of the sampled women according to their socio-demographic factors. Overall, the mean levels of all the six elements except selenium were higher in the hair specimens than in nails. The mean ± SD levels of Cr, Zn, Se, Cu, Fe and Mn in hair specimens were 3.30 ± 0.56, 71.27 ± 8.92, 0.35 ± 0.08, 16.30 ± 4.39, 35.42 ± 5.44 and 1.92 ± 0.13, respectively. The mean levels of Zn and Se were significantly low among working participants compared to non-working ($p<0.05$). Similarly, the mean levels of Fe and Mn among working participants were lower than the levels among non-working. However, the difference between the two groups was statistically not significant ($p>0.05$). According to diabetes control, the mean level of Cu among those with poor control was significantly lower than the levels among the women with good control ($p<0.05$). However, there were no significant differences in the mean levels of other elements in the hair specimens according to the age, number of children, educational level, employment status, diabetes control and duration of diabetes. On the other hand, the mean ± SD levels of Cr, Zn, Se, Cu, Fe, and Mn in nails specimens were 2.75 ± 0.66, 60.55 ± 3.93, 0.50 ± 0.16, 4.30 ± 0.39, 24.42 ± 2.64 and 0.29 ± 0.03, respectively. From Table 3, only the Cu level showed a significant difference between participating women according to diabetes control; those

TABLE 3. Mean levels of trace elements in hair specimens of the subjects

Variables	Trace elements levels in hair [mean (µg/g) ± SD]					
	Cr	Zn	Se	Cu	Fe	Mn
Age [a]						
20-30	2.92 ± 0.48	62.51 ± 13.12	0.28 ± 0.05	19.26 ± 5.50	49.55 ± 6.31	2.35 ± 0.13
31-40	3.67 ± 0.74	75.62 ± 12.32	0.39 ± 0.08	16.42 ± 4.00	22.18 ± 6.62	2.11 ± 0.22
41-50	3.16 ± 0.14	81.82 ± 11.73	0.31 ± 0.06	17.52 ± 6.24	35.52 ± 6.72	1.94 ± 0.11
51-60	3.19 ± 0.52	58.90 ± 12.22	0.47 ± 0.05	14.49 ± 6.84	49.66 ± 4.62	1.62 ± 0.32
Education status [a]						
Primary school	3.33 ± 0.44	83.92 ± 14.41	0.35 ± 0.12	19.77 ± 2.21	43.33 ± 8.11	2.36 ± 0.45
Secondary school	3.35 ± 0.54	72.09 ± 11.07	0.39 ± 0.21	15.20 ± 3.73	34.38 ± 7.12	1.87 ± 0.97
Diploma/STPM	2.97 ± 0.23	72.44 ± 10.31	0.29 ± 0.08	18.07 ± 3.71	29.90 ± 6.53	1.67 ± 0.29
Employment status [b]						
Working	3.45 ± 0.58	64.98 ± 11.10*	0.30 ± 0.18*	17.38 ± 4.83	33.92 ± 6.16	1.81 ± 0.14
Not working	3.12 ± 0.49	84.91 ± 10.32	0.42 ± 0.15	16.39 ± 3.11	41.22 ± 5.12	2.02 ± 0.15
Number of children [b]						
<4	3.07 ± 0.55	76.87 ± 10.24	0.37 ± 0.08	15.49 ± 5.17	32.69 ± 4.81	2.03 ± 0.28
≥4	3.41 ± 0.59	68.67 ± 9.34	0.36 ± 0.07	18.15± 6.30	30.23 ± 4.21	1.83 ± 0.07
Diabetes control [b]						
Good	3.77 ± 0.36	63.48 ± 10.35	0.30 ± 0.05	20.41 ± 4.20	35.33 ± 7.31	1.66 ± 0.06
Poor	3.07 ± 0.34	77.45 ± 8.42	0.38 ± 0.08	15.67 ± 4.31*	36.63 ± 5.72	2.05 ± 0.18
Duration of diabetes [b]						
≤5 years	3.40 ± 0.55	70.30 ± 12.32	0.37 ± 0.06	17.68 ± 5.22	34.60 ± 5.63	1.88 ± 0.34
>5 years	3.10 ± 0.66	72.94 ± 13.68	0.31 ± 0.03	15.03 ± 4.01	37.19 ± 4.16	1.97 ± 0.28

[a] Differences were examined by one-way ANOVA; [b] differences were examined by independent t-test
* Significant difference compared to poor control group (*p*<0.05)

TABLE 4. Mean levels of trace elements in nails specimens of the subjects

Variables	Trace elements levels in nails [mean (µg/g) ± SD]					
	Cr	Zn	Se	Cu	Fe	Mn
Age [a]						
20-30	3.31 ± 0.07	68.34 ± 5.22	0.46 ± 0.16	3.42 ± 0.37	22.03 ± 2.03	0.20 ± 0.04
31-40	3.29 ± 0.21	58.94 ± 4.52	0.46 ± 0.21	3.93 ± 0.64	23.25 ± 2.15	0.37 ± 0.04
41-50	2.35 ± 0.08	59.46 ± 9.60	0.50 ± 0.15	4.40 ± 0.40	25.44 ± 3.34	0.28 ± 0.03
51-60	2.64 ± 0.12	60.85 ± 4.96	0.57 ± 0.15	5.09 ± 0.79	24.37 ± 6.09	0.27 ± 0.06
Education status [a]						
Primary school	2.62 ± 0.71	61.96 ± 3.10	0.44 ± 0.07	3.65 ± 0.53	26.17 ± 5.68	0.23 ± 0.02
Secondary school	2.30 ± 0.78	60.00 ± 1.74	0.50 ± 0.04	4.38 ± 0.39	24.41± 3.13	0.31 ± 0.03
Diploma/STPM	3.30 ± 0.92	61.03 ± 2.76	0.56 ± 0.04	5.13 ± 0.63	23.44 ± 3.53	0.28 ± 0.06
Employment status [b]						
Working	2.62 ± 0.64	59.58 ± 4.90	0.50 ± 0.16	4.56 ± 0.14	24.11 ± 2.41	0.33 ± 0.03
Not working	2.79 ± 0.67	61.63 ± 3.12	0.50 ± 0.17	4.18 ± 0.42	24.70 ± 3.80	0.25 ± 0.02
Number of children [b]						
<4	2.45 ± 0. 13	59.50 ± 2.61	0.48 ± 0.04	4.10 ± 0.35	24.61 ± 2.23	0.29 ± 0.03
≥4	3.06 ± 0.49	61.12 ± 2.07	0.53 ± 0.04	4.69 ± 0.50	22.99 ± 3.57	0.28 ± 0.04
Diabetes control [b]						
Good	3.11 ± 0.75	60.13 ± 3.42	0.50 ± 0.06	3.35 ± 0.46*	19.59 ± 3.18	0.24 ± 0.03
Poor	2.56 ± 0.51	60.70 ± 2.28	0.51 ± 0.03	4.69 ± 0.33	26.09 ± 2.59	0.30 ± 0.03
Duration of diabetes [b]						
≤5 years	2.81 ± 0.31	60.71 ± 9.12	0.55 ± 0.10	4.55 ± 0.62	21.63 ± 2.61	0.30 ± 0.03
>5 years	2.54 ± 0.33	58.23 ± 5.46	0.48 ± 0.07	4.84 ± 0.65	28.10 ± 2.14	0.32 ± 0.04

[a] differences were examined by one-way ANOVA; [b] differences were examined by independent t-test
* significant difference compared to poor control group (*p*<0.05)

with poor control had lower Cu levels ($p<0.05$). However, there were no significant differences in the mean levels of other elements in the nails specimens according to age, number of children, educational level, employment status, diabetes control and duration of diabetes.

Pearson's correlation coefficients for the levels of trace elements in the hair and nails specimens are shown in Table 5. There were significant and positive correlations between Cr/Cu, Zn/Mn and Se/Mn levels in the hair specimens (r=0.63, 0.43 and 0.32, respectively). Similarly, there were significant positive correlations between Cr/Zn, Fe/Mn and Zn/Fe (r=0.31, 0.47 and 0.30). On the other hand, there was a significant negative correlation between Se and Mn (r=- 0.30). The correlation between trace elements in hair and nails specimens was investigated and found to be not significant ($p>0.05$).

DISCUSSION

The prevalence of diabetes mellitus is increasing in developing countries, including Malaysia, with a growing incidence of type 2 DM which accounts for about 90% of all cases. Alteration in the status of trace elements in diabetic patients has been reported with elements deficiencies as an evident disturbance of trace elements in diabetes (Khalid et al. 2014). Data on trace elements status among Malaysians is still lacking. Few studies have been done in Asia to evaluate the status of certain trace elements among diabetes mellitus patients (Alwan & Hamood 2017; Atieh et al. 2015; Praveeena et al. 2013; Skalnaya & Demidov 2007; Sukumar & Subramanian 2007; Tasneem et al. 2008; Yerlikaya et al. 2013). Moreover, data on the status of trace elements in the nails of diabetic patients are still limited.

In the present study, the levels of six elements were evaluated in the hair and nails specimens of Malay women with type 2 DM. The levels of these elements, except Se, were higher in the hair samples compared to the nails. The findings showed that Cr levels in nails and hair were more than the values reported in India and Pakistan (Sukumar & Subramanian 2007; Tasneem et al. 2008). Taking into consideration the influence of many factors like occupation, dietary habits, climate, geographical location, parameters of trace element exchange may alter between populations (Skalnaya & Skalny 2018). The higher values of Cr among Malay women could be attributed to the consumption of some foods such as nuts, mushrooms and oysters which are essential in the Malaysian cuisine. These kinds of foods are rich in Cr (Gibson 1998). The ingestion of supplements and traditional medicine is a possible cause since some respondents mentioned using these compounds.

A previous local study reported the availability of Cr besides 28 other elements in the medicinal herbs commonly used in Malaysia (Sarmani et al. 1999). Additionally, a recent report in Malaysia found that 52.4% of diabetic patients with type 2 DM use dietary supplements (Tan et al. 2015). Variation in Cr and other trace elements measurements due to the instruments, sample collection, washing procedures, age and gender should also be considered.

Regarding the levels of Zn in hair and nails, our findings showed lower levels among Malay women compared to previous studies abroad. This might be explained by the variation in environment and food consumption (Tasneem et al. 2008). Malaysians eat less red meat which is the major source of bioavailable Zn (Tran 2008). The mean level of Cu in the hair samples reported by

TABLE 5. Correlation analysis of trace element in hair and nails specimens of the subjects

Trace element in pairs	Hair specimens		Nails specimens	
	r[a]	P	r[a]	P
Cr/Zn	0.016	0.461	0.316*	0.025
Cr/Se	0.191	0.122	0.199	0.112
Cr/Cu	0.550**	0.001	-0.024	0.441
Cr/Fe	0.208	0.154	-0.008	0.481
Cr/Mn	- 0.148	0.185	0.149	0.183
Zn/Se	0.184	0.131	0.082	0.309
Zn/Cu	0.020	0.452	0.107	0.259
Zn/Fe	0.120	0.280	0.307*	0.029
Zn/Mn	0.467**	0.001	0.066	0.345
Se/Cu	- 0.168	0.153	0.153	0.176
Se/Fe	0.189	0.177	- 0.258	0.056
Se/Mn	0.382**	0.008	- 0.309*	0.028
Cu/Fe	- 0.014	0.474	0.161	0.164
Cu/Mn	0.060	0.359	0.163	0.160
Fe/Mn	0.113	0.291	0.474**	0.001

a Pearson's correlation coefficient
* Significant at $p<0.01$; ** Significant at $p<0.001$

the present study was higher than the mean level reported among Pakistani patients (Tasneem et al. 2008). However, Sukumar and Sumramanian (2007) reported a higher level among Indian patients. Our study found no significant difference in the levels of trace elements according to age groups. This finding supports previous reports from India and Bangladesh (Hussain et al. 2009; Sukumar & Subramanian 2007).

In contrast, Tasneem et al. (2008) reported that the levels of Zn and Cr in blood and hair scalp were low in the older diabetic subjects compared to the younger patients. This could be attributed to the lower absorption of nutrients through the gut in the elderly subjects. Also, elderly people eat less and due to the difficulty of leaving the house, could be more likely to eat long-life food, low in Zn and some nutrients (Garg et al. 2005). Moreover, Sánchez et al. (2010) reported a positive correlation between plasma Se and age. It was postulated that there is a tendency for Se levels to increase among the population with a stronger preference for a traditional diet consumed mainly by elderly people more than younger groups (Sánchez et al. 2010).

The present study found no significant differences in the levels of trace elements among the subjects according to their educational level and number of children. All subjects had primary school education as a minimum, which means all patients have a basic degree of education. Persell et al. (2004) concluded that knowledgeable patients were more likely to perform self-management activities.

Some studies showed that serum Zn levels decreased in diabetic patients (Bolajok et al. 2017; Praveena et al. 2013). The present study showed significant differences in the levels of Zn, Se, and Cu according to the employment status of subjects. The levels of Zn and Se in the hair specimens were low among working compared to non-working women, possibly due to the occupational stress. Stress has been reported to affect the metabolism of Zn resulting in a reduction in plasma zinc levels and enhancing Zn loss in the urine (Boosalis et al. 1991). Moreover, stress led to an internal redistribution of Zn to other organs such as the liver and loss from other tissues such as the intestine and pancreas (Heyland et al. 2008; McClain et al. 1993). Interestingly, a recent meta-analysis reported possible zinc deficiencies in type 2 diabetic individuals (Namrata et al. 2018).

Previous studies concluded that the exposure to chronic psychological stressors such as job pressures or psychological stress caused by the death of a spouse or a financial crisis is associated with higher risk of developing diabetes in middle age and contribute to the global burden of the disease (Wellen & Hotamisligil 2005: WHO 2001). The lower levels of Se in the hair of the working group may be attributed to the increased need for selenium due to the exposure to the polluted environment in some workplaces.

A previous study showed that plasma selenium levels among oil refinery workers were significantly lower than their levels among non-industrial groups. Furthermore, water quality, hair treatment and cosmetics such as some shampoo formulated with selenium sulfide might also contribute to the variation of the Se levels (LeBlanc et al. 1999). Interestingly, our findings variation in the levels of Cu in the hair and nails specimens according to the employment status of the subjects. We found that the level of Cu in the hair specimens was significantly lower among women with good or satisfactory diabetes control compared to those with poor diabetes control.

In contrast, the level of Cu in the nail's specimens of women with poor diabetes control was significantly higher than in those with good diabetes control. Previous studies reported that concentrations of trace elements were not related to the degree of glucose control as determined by correlation analysis between HbA1c and elements levels in the blood fractions (Ekmekcioglu et al. 2001; Rohn et al. 1993). We believe that the result using hair specimens was more acceptable as the lower Cu in poor diabetes control group associated with higher Zn levels. Rowin and Lewis (2005) and Willis et al. (2005) reported that Zn could replace Cu in people using supplements or minerals containing excess Zn. Moreover, elements levels in nails are mainly influenced by place of residence whereas hair elements are influenced by seasonal variations (Wilhelm et al. 1991). Glycated haemoglobin (HbA1c) estimates blood glucose concentration for the previous two to three months, and nails and hair samples showed elements concentration for the long-term (Jun et al. 1990). Thus, using HbA1c as an indicator of diabetes control to correlate or to compare between trace elements in hair and nails might not be useful unless only one or two proximal centimetres of hair are used. The increase in Cu levels in diabetic patients might be attributed to the hyperglycaemia that may stimulate glycation to release Cu ions from the copper contains enzymes and proteins such as superoxide dismutase (SOD) and ceruloplasmin (CP) (Abou-Seif & Youssef 2004).

In the present study, the duration of diabetes did not influence the trace elements levels. This was consistent with previous studies (Hussain et al. 2009; Zargar et al. 1998). Additionally, a recent report showed that as the duration of DM increases, the Cr level was decreased, whereas no statistically significant change was shown in the levels of Mn, Zn, Fe and Cu (Gahlot & Bhatnagar 2018). However, Nsonwu et al. (2006) reported that serum and urine trace elements concentrations varied significantly with the increasing duration of diabetes urging that diabetes alters the metabolism of zinc, magnesium, selenium and chromium by decreasing their serum concentration and increasing their urinary excretion (Nsonwu et al. 2006).

Our findings showed significant correlations between the elements taking into consideration the type of specimen. These findings support previous studies (Ömer et al. 1993; Sukumar & Subramanian 2007). Watts (1990) urged that the relationship between nutrients is complex, especially between trace elements. Thus, an affected element will affect at least two minerals, and each of them will also affect two others. Moreover, some elements act as synergism or antagonism to others.

Although this study is the first, to the best of our knowledge, to evaluate the status of trace elements among diabetic patients in Malaysia, some limitations in the study design (cross-sectional) should be considered. These include its small sample size and absence of control due to limited study time, lack of serum samples and urine samples, lack of dietary habits history, data on the taking of supplements was not available due to the difficulty to recall or to know the contents of the elements contained in the supplements.

In conclusion, the findings of this study indicate that there were differences in the levels of trace elements between hair and nails among Malay women with type 2 diabetes mellitus. However, socio-demographic factors could influence the levels of certain trace elements. To better understand the role of these trace elements in diabetes, more studies are needed. The results could also be supported by a more significant number of patients and by using blood and urine samples.

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