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The relationship between telomere length and beekeeping among Malaysians

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Abstract The belief that beekeepers live longer than anyone else is present since ages. However, no research has been done to explore the longevity of life in beekeepers. Here, we investigated the telomere length in 30 male beekeepers and 30 male non-beekeepers and associated them with the longevity of life using Southern analysis of terminal restriction fragments (TRFs) generated by Hinf I/Rsa I digestion of human genomic DNA using *Telo*TAGGG Telomere Length Assay. Interestingly, we found that the telomere length of male beekeepers was significantly longer than those of male non-beekeepers with a p value of less than 0.05, suggesting that beekeepers may have longer life compared to non-beekeepers. We further found that the consumption of bee products for a long period and frequent consumption of bee products per day are associated with telomere length. An increase of year in consuming bee products is associated with a mean increase in telomere length of 0.258 kbp. In addition, an increase in frequency of eating bee products per day was also associated with a mean increase of 2.66 kbp in telomere length. These results suggested that bee products might play some roles in telomere length maintenance.

Keywords Longevity · Beekeeping · Telomere · Southern blot

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Introduction

> There is nothing in the world that could beat honey as an aid to defy old age. Keep bees and eat honey if you want to live long. Beekeepers live longer than anybody else.
> - John Anderson

Bees have been of human interest for over 5000 years due to the benefits of honey (Association 2005). Ancient Egypt, for example, highly valued the honey and bees. Since the earliest times, one of the pharaohs' titles was Bee King and the Gods were also associated with bees. In addition, bees were also chosen as a symbol for the

country. They kept bees and honey in temples and named them as Mansion of Bee (Crane 1999). These events suggest long existence of beekeeping activities.

Interestingly, honey has been proposed as an important food item in human evolution (Crittenden 2011; Wrangham 2011). Recently, it is thought that the ability of human to climb trees mainly stems from the desire to collect honey (Kraft et al. 2014). Honey is extremely energy-dense (~3.0 kcal g^{-1}) and nutritious (Bogdanov et al. 2008). Besides that, it has many functional properties desired by humans such as long preservation time (Nagai et al. 2006) and antimicrobial, antiviral, antiparasitic, anti-inflammatory and antioxidant effects (Bogdanov et al. 2008). Other bee products such as propolis and royal jelly are also widely known for such properties (Viuda-Martos M et al. 2008). Hence, it is not surprising that bee products could play a vital role in human evolution.

There are many instances in history which confirm the belief that beekeepers seemed to live longer than anyone else. Examples include François Huber who lived until he was 81 years old, Lorenzo Lorreine Langstroth who died at 85 years old and Johann Dzierzon who lived until he was 95 years old (Health 2014). Even so, there is still a dearth of research and information to explore if this belief is only the 'old wives tales' or vice versa.

Telomeres are the tandem repeat sequence of TTAG GG (Blackburn 1991; Lu et al. 2013) and associated with telomere-associated proteins called shelterin (Lu et al. 2013). Telomeres shorten with every cell division (Harley et al. 1990) as the DNA replication machinery is unable to copy the ends of the linear molecules (Olovnikov 1970). Telomere length has been suggested to be a marker of biological ageing (Mather et al. 2011). In addition, shorter telomere length has been associated with ageing as well as human ageing-associated diseases like cancer, cardiovascular diseases and obesity (Blackburn 2010; Codd et al. 2013). In this connection, telomere length can be a good indicator of measuring the longevity of life biologically.

Bearing that in mind, this research aims to provide an insight into the longevity of life in beekeepers by measuring and comparing the mean terminal restriction fragment length (TRF) between beekeepers and non-beekeepers and associating them with longevity of life. Besides that, we hope to shed some light on the factors that may influence the longevity of life in beekeepers.

Materials and methods

Ethical approval to conduct this study was granted by the Human Research Ethics Committee Universiti Sains Malaysia, Health Campus, Malaysia, as per reference USMKK/PPP/JEPeM [241.3.(3)]/Amend (01) dated 29th September 2011.

Subjects were recruited from all over Malaysia in 2012 based on our selection criteria. The inclusion criteria included healthy male beekeepers and non-beekeepers above 30 years of age, beekeepers who were associated with beekeeping for a minimum period of 5 years and non-beekeepers who did not consume any bee products and were not associated with beekeeping and related activities. The exclusion criteria comprised beekeepers and non-beekeepers with any disease and/or any kind of medication. Upon blood sample collection, blood pressure was measured and recorded. All subjects signed an informed consent approved by the Human Research Ethics Committee, Universiti Sains Malaysia.

Mean TRF length was measured using *Telo*TAGGG Telomere Length Assay (Roche, Germany) according to the provided protocol. The DNA was extracted from blood samples using DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's instructions. For DNA digestion, 1.5 μg of DNA was digested with restriction enzymes provided in the kit, Rsa I and Hsa I for 2 h at 37 °C. Then, the digested DNA was subjected to 0.8 % agarose gel electrophoresis at 75 V for 4 h. The Southern blot apparatus was assembled after depurination, denaturation and neutralization processes. The gel was blotted on positively charged nylon membrane (Roche, Germany) overnight at room temperature. On completion of blotting, the transferred DNA was fixed on wet membrane by UV-crosslinking for 20 min at 120 mJ. The blot was then subjected to hybridization and washing as described in the manual. The mean TRF length was obtained by comparing signals relative to a nuclear weight standard on an X-ray film. The chemiluminescence signals were quantified using ImageJ software (National Institute of Health, USA).

The calculation of mean TRF length was defined as mean $\mathrm{TRF} = \sum(\mathrm{OD}_i) \Big/ \sum\left({}^{\mathrm{OD}_i}/_{L_i}\right)$ where OD_i is the chemiluminescent signals and L_i is the length of TRF at position i.

Data evaluation and statistical analysis of independent t test and multiple regression were performed using SPSS Statistics Version 22 software. The result was considered significant if p value was less than 0.05.

Results

The mean (SD) baseline characteristics for the beekeepers (n=30) and non-beekeepers (n=30) are shown in Table 1. The baseline characteristics were similar in both groups in terms of age and blood pressure.

Table 2 shows the mean TRF length for both beekeepers and non- beekeepers. The group of beekeepers had a mean of 8.13 kbp with SD 3.64 which was longer than the non-beekeeper group who had a mean of 5.85 kbp with SD 1.53. The results of independent t test showed that the difference between the mean TRF length of both groups was significant, with p value <0.05.

We further examined the association between age, period of beekeeping, number of bee products consumed, period of bee product consumption and frequency of eating bee products per day with telomere length. Results are shown in Table 3. When we combined the predictors in both groups, we found that the period of bee products' consumption and frequency of eating bee products per day were independently predictive of increasing telomere length. An increase of year in consuming bee products is associated with a mean increase in telomere length of 0.258 kbp. In addition, an increase in frequency of eating bee products per day was also associated with a mean increase of 2.66 kbp in telomere length. However, age, period of beekeeping and number of bee product consumption did not show any association with telomere length (p>0.05).

Discussion

Our study found that beekeepers have significantly longer telomere length as compared to non-beekeepers. In addition, we also found that longer period of consumption of bee products is associated with longer telomere length. Besides that, an increase in frequency of bee products' consumption per day was associated with longer telomere length as well. To the best of our knowledge, this is the first report of its kind.

Telomeres are important for chromosome integrity and are maintained by an enzyme called telomerase (Lu et al. 2013). Telomere maintenance is crucial because it provides protection against various DNA damage response (DDR) which include ataxia telangiectasia-mutated gene (ATM) and ATM and Rad3-related (ATR) pathways homologous recombination (HR) and non-homologous end joining (NHEJ) (de Lange 2009) which could lead to replicative senescence and contribute to ageing (Shay and Wright 2007). Besides ageing, telomere length is proposed to be associated with longevity of life as well. Studies in premature ageing syndromes like Werner, Hutchinson-Gilford and dyskeratosis congenita (DC) display the traits of shorter telomeres, accelerated ageing and reduced life span (Kirwan and Dokal 2009). In addition, the introduction of telomerase into normal human cells has been shown to extend the life span of the cells by maintaining the telomere length without provoking malignant properties (Bodnar et al. 1998; Counter et al. 1998). On the other hand, longer telomeres have been positively associated with healthy life and longevity (Atzmon et al. 2010). Those individuals with longer telomeres have an overall improved health profile and better cognitive functions and lipid profiles as compared to controls. Consistently, several studies in human populations managed to highlight the negative correlation of telomere length with age (Atzmon et al. 2010; Njajou et al. 2007, 2009). These evidences therefore suggested that longer telomere length might reflect longevity of life.

Telomere length is influenced by few factors (Saliques et al. 2010; Stewart et al. 2012). However, oxidative stress is one of the key contributors to the telomere shortening due to the telomere structure itself (Kawanishi and Oikawa 2004). Telomere sequence for

Table 1 Mean (SD) baseline characteristics for beekeepers and non-beekeepers

Characteristic		Group	Mean (SD)	Mean difference (95 % CI)	t statistic (df)	p value
Age		Beekeepers (n=30) Non-beekeepers (n=30)	44.37 (9.503) 41.03 (7.79)	3.33 (−1.58, 7.83)	1.49 (58)	>0.05
Blood pressure (mmHg)	Systolic	Beekeepers Non-beekeepers	137.33 (19.64) 137.69 (17.67)	−0.36 (−10.02, 9.30)	−0.75 (58)	>0.05
	Diastolic	Beekeepers Non-beekeepers	76.47 (11.85) 80.77 (16.65)	−4.30 (−11.77, 3.17)	−1.15	>0.05

Table 2 Difference between mean TRF length among beekeepers and non-beekeepers

Group	Mean (SD)	Mean difference (95 % CI)	*t* statistic[a] (df)	*p* value
Beekeepers (n=30)	8.13 (3.64)	2.28 (0.82, 3.74)	3.16 (38.91)	<0.05
Non-beekeepers (n=30)	5.85 (1.53)			

[a] Independent *t* test was applied

example contains many guanines which are prone to be oxidized to 8-oxod-G by reactive oxygen species (ROS). Besides that, progressive increase in 8-oxod-G has been shown to be associated with decreasing telomere length (Kawanishi and Oikawa 2004). In addition, ROS, especially the hydroxyl radical, induce breaks in DNA and deteriorate DNA base repair (Fotiadou et al. 2004). Telomere seems to be unable to repair the DNA breaks as compared to the rest of the genome (Petersen et al. 1998) because of the binding of the TRF2 on the telomere sequence which prevents the DNA repair enzymes from reaching the site (Richter et al. 2007). It is also thought that the GGG-specific DNA damage in telomere sequence induced by oxidative stress may play an important role in increasing the rate of telomere shortening (Oikawa and Kawanishi 1999; Oikawa et al. 2001).

Bee products such as honey, royal jelly and propolis are widely known for their great antioxidant capacity (Viuda-Martos et al. 2008). Honey consists of up to 95 % carbohydrates (Bogdanov et al. 2008) and includes an extensive selection of proteins, enzymes, amino acids, minerals, trace elements, vitamins and polyphenolic compounds (Bogdanov et al. 2008; Alvarez-Suarez et al. 2010; Aparna and Rajalakshmi 1999). Antioxidant capacity of honey and propolis is thought to be mainly contributed by the phenolic compounds and flavonoids in them (Aljadi and Kamaruddin 2004). A study conducted showed that honey exhibited protective effect in endothelial cells against oxidative stress. They further demonstrated that honey components could prevent the atherogenic action of oxidized LDL and boost the intracellular GSH pool which plays a key role in counteracting the action of circulating ROS and reactive nitrogen species (RNS) (Beretta et al. 2007).

Table 3 Multiple regression results for relative telomere length

Predictor	Coefficient (95 % CI)[a]	*p* value
Age	−0.038 (−0.12 to 0.047)	0.371
Period of beekeeping (years)	−0.171 (−0.37 to 0.02)	0.085
Number of bee products consumed	−0.483 (−1.17 to 0.2)	0.16
Period of bee product(s) consumption (years)	0.258 (0.07 to 0.44)	0.007
Frequency of eating bee product(s) (per day)	2.66 (0.16 to 5.16)	0.038

[a] Each one unit change in the predictor indicates an increase or decrease in relative telomere length (kbp) by the amount of coefficient with other predictors held constant

The significant effect of bee products' consumption in the long run is as predicted. Although the main mechanisms on how bee products protect telomere length in beekeepers are unknown, we presume that bee products provide protective effects on telomere length against oxidative stress. Increase in frequency of bee products' consumption per day could probably add more protective effects on telomere. While little is known about the influence of bee products on telomere length, telomere length has been shown to be associated with nutritional status in human and animal studies (Paul 2011). Healthy lifestyles and diets are positively correlated with telomere length (Ornish et al. 2013). Additionally, antioxidant properties of vitamin C and E (Honarbakhsh and Schachter 2009) are also positively associated with longer telomeres in a dose-dependent manner in women (Xu et al. 2009). Other than that, the addition of physiological concentrations of vitamin C or vitamin E to the culture medium could slow down the age-dependent shortening of telomeres as well as decrease in telomerase activity in cell cultures, hence increasing the life span (Furumoto et al. 1998; Yokoo et al. 2004; Tanaka et al. 2007). In cells treated with vitamin E (6-O-phosphorylated form of α-tocopherol), there was a reduction in the ROS due to scavenging by the vitamin (Tanaka et al. 2007). This process may limit oxidative damage to telomeric DNA that would otherwise cause shortening of telomere length. Changes in diet and lifestyle can also control telomerase activity in peripheral blood mononuclear cells (Ornish et al. 2008). The effect of bee product consumption on telomerase activity is however beyond the scope of this study.

To our surprise, the period of beekeeping does not influence telomere length when it is expected that it may do so. Perhaps, this evidence supports the results that greater telomere length of beekeepers is primarily due to the longer period of consumption of bee products and the frequency of eating bee products per day. Although we could find association between telomere length and period of bee product consumption and the frequency of bee product consumption per day, this study, however, could not determine both the type of bee products as well as the frequency of consumption of bee products that may influence the telomere length the most. Besides that, our study is also limited to cross-sectional nature. However, our telomere analyses method which employed Southern blot has added strength to this study as it is termed as the gold standard in telomere research.

In conclusion, our study has five main findings. Firstly, telomeres in beekeepers are longer as compared to non-beekeepers based on statistical significance analysis. Secondly, since telomere length reflects the longevity of life, biologically, this means that beekeepers may have longer life as compared to non-beekeepers. Thirdly, longer telomere length in beekeepers is associated with longer period of bee product consumption. Other than that, frequent consumption of bee products per day is also associated with longer telomere length. Finally, this study suggests that bee product consumption might be beneficial to telomere length maintenance. Further research on a larger cohort study is deemed necessary to support our findings which is also crucial to provide a better understanding in the roles played by bee products in telomere biology, thus offering a new pathway for us to manage ageing and telomere-associated diseases.

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Conflict of interest The authors declare that they have no competing interests.

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