Short Communication

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Salivary testosterone as a potential indicator for risky behaviour associated with smoking-related peer pressure in adolescents

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Abstract: Early smoking is considered an indicator for risky behaviour in adolescents. Although social indicators predicting adolescent smoking are known, biological indicators have not been defined. This study aimed to establish whether salivary testosterone could be used as a "predictive biomarker" for smoking-associated peer pressure. Saliva samples were collected from Bruneian adolescents (aged 13–17 years) by the passive drool method. Salivary testosterone concentration was determined by enzyme-linked immunosorbent assay. Salivary testosterone concentration and smoking-associated peer pressure indicators were compared between adolescent males and females and statistical significance was determined by an independent samples t-test. A significant positive relationship between smoking-associated peer pressure and salivary testosterone levels in adolescents was found. However, this relationship was not significant when males and females were considered separately. Our data suggest that students who have tried cigarette smoking and have friends who are cigarette smokers have higher salivary testosterone levels.

Keywords: adolescents; behaviour; saliva; smoking; testosterone.

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Introduction

Risky behaviour in adolescents is a concern to parents, law enforcement and the general public. One of the early social indicators for risky behaviour in adolescents is early smoking. Early risky behaviour shapes adult behaviour, and the consequences are costly to society and young people. In addition to sociological indicators, biological indicators of risky behaviour also provide essential information to support the development of effective preventative measures against the onset of risky behaviour. The idea of using salivary testosterone as a putative biomarker to predict risky behaviour associated with smoking was first proposed in the late 1980s (1). Testosterone is a major male sex hormone, but it is also present in women at much lower concentrations. Testosterone is easily measured in saliva and has a diurnal rhythm characterised by a steady decrease during waking hours (2). Furthermore, testosterone undergoes shorter-term fluctuations in response to challenge (e.g. emotional stress) during adolescence and is affected by environmental stimuli from adolescence to adulthood. To date, limited work has been done to determine the relationship between salivary testosterone and smoking in adolescents (3), and few studies have considered these factors in relation to smoking-associated peer pressure (1). To further understand this connection, we assessed the relationship between salivary testosterone levels and smoking-associated peer pressure as an indicator of risky behaviour in adolescents.

Materials and methods

Ethical approval

This project received ethical clearance from the PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam Ethics Committee and the Ministry of Education, Darussalam, Brunei.

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Subjects

Multistage cluster sampling involving secondary schools in the Brunei-Muara district, Brunei Darussalam, was used. The calculated sample size was 353 students, with an expected attrition rate of 20%. Students were asked to complete questionnaires, and saliva samples were then collected. No physical examination was performed during data collection. Informed consent was obtained from all individual participants, school headmasters and their parents. Inclusion criteria encompassed students without any communicable or non-communicable diseases. Students were advised not to eat or consume carbonated drinks 1-h prior to saliva collection.

Saliva collection

Saliva was collected by trained research personnel using the passive drool technique as described in the guidelines for Salimetrics saliva collection methods (Salimetrics, LLC, State College, PA, USA). Participants were required to drool into a 15-mL sterile centrifuge tube to give a volume of approximately 1 mL. Saliva was centrifuged at 780 *g* for 15 min before storing at −80 °C.

Salivary testosterone concentration measurement

Saliva was thawed at room temperature before duplicate testosterone analyses using enzyme-linked immunosorbent assay (ELISA). The concentration of salivary testosterone (pg/mL) was measured using the Testosterone Salivary Immunoassay Kit (Salimetrics, LLC) according to the manufacturer's specifications.

Statistical analysis

Salivary testosterone concentration and smoking-associated peer pressure indicators were compared between adolescent males and females and statistical significance was determined by an independent samples *t*-test. Data were analysed using IBM SPSS Version 21.0 (SPSS Institute, Chicago, IL, USA).

Results

Six of the nine sampled schools participated, resulting in an overall school response rate of 66.7%. Out of the remaining students to be sampled, only 121 had parental consent. Of these, five students refused to participate, and 23 students were absent on the day of data collection, resulting in distribution of only 93 questionnaires. However, a further six questionnaires were discarded for being under the minimum target age for participation in this study (13-years-old). Therefore, 87 out of 93 questionnaires were usable (response rate: 93.5%). The total student response rate from the schools was 62.4%. Sixteen of the 87 saliva samples were excluded from analysis based on a poor percentage coefficient of variation (CV) (>10% CV). Therefore, 71 samples (26 males and 45 females aged 13–17 years) were selected for analysis. Of these students, 22.5% (16/71) had tried smoking cigarettes, and the mean salivary testosterone level for students who had tried smoking was significantly greater than that of those who had not (p=0.033) (Table 1). The mean salivary testosterone level for students who had friends that smoke cigarettes (50/71) was significantly greater than those who did not (p=0.018) (Table 1). When males (p=0.916 for "Have tried smoking cigarettes" and p=0.491 for "Had friends that smoked cigarettes") and females (p=0.069 for "Have tried smoking cigarettes" and p=0.061 for "Had friends that smoked cigarettes") were considered separately (Table 1). The observed difference was not significant, but average salivary testosterone levels were higher in female students who had friends who smoked cigarettes than in those who did not.

Discussion

We found a significant positive relationship between smoking-associated peer pressure and salivary testosterone levels in adolescents. This agrees with earlier findings that show a strong correlation in 12–14-year-old males, but not females, between high testosterone serum levels and cigarette smoking due to peer pressure (1). However, we did not see a significant difference when the male and female data were analysed separately, although a clear difference in mean values showed otherwise (Table 1). We attributed this to low sample numbers. It is possible that the differences in basal levels and diurnal patterns for salivary testosterone between boys and girls (4) could also account for this difference. However, this is unlikely as the

Table 1: Relationship between salivary testosterone levels (pg/mL) and smoking-related peer pressure.

	Yes n (mean±SD)	No n (mean±SD)
Tried cigarettes		
All	16 (115.0±42.8)	55 (88.2±43.4)
Male	9 (132.2±28.4)	17 (130.4±44.9)
Female	7 (92.8±49.9)	38 (69.3±26.3)
Have friends who smoke		
All	50 (102.2±45.5)	21 (75.1±35.9)
Male	21 (133.7±41.5)	5 (119.9±29.1)
Female	29 (79.5±33.4)	16 (61.1±24.8)

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time of saliva collection in our study was standardised to avoid the diurnal variation factor. A study by Urberg and Liang (5) found that smoking adolescents appeared to see the peer group, not as encouraging them to smoke, but as not providing any discouragement. Therefore, as in a previous study (3), we made a similar assumption that friends who smoked could have a peer-pressure effect on others to smoke. Hence, why we used this as a fitting indicator for smoking-associated peer pressure in our study. Because high endogenous testosterone levels have been shown to be related to increased risk-taking behaviour in boys and girls (6), it is possible that adolescents with high testosterone levels may actively seek out risk-taking opportunities. The possibility of using salivary testosterone as a biological indicator for risky social behaviour should be explored in prospective studies. However, it is important to consider interdependent hormonal systems simultaneously rather than focusing on a single hormone.

In conclusion, students who have tried cigarette smoking and have friends who are smokers have higher salivary testosterone. Future work should involve exploring relationships between salivary testosterone and other social indicators of risky behaviour (e.g. drug-taking habits) and investigating potential interactions that testosterone may have with other hypothalamic-pituitary-gonadal (HPG) or hypothalamic-pituitary-adrenal (HPA) hormones. Because the HPA and HPG endocrinal axes are highly interactive (2), examining both systems in future studies may better predict the appearance of risky behaviour in adolescence compared with considering each axis individually. The inclusion of psychological variables in future studies of risk-taking behaviour would be an important addition to biological and social data.

Our study suggests that salivary testosterone may be a useful biological marker for predicting early smoking behaviour in adolescents. Data from this study will hopefully provide insights into patterns of the onset of early adolescent smoking, for which preventative measures can be devised.

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Conflict of interest statement: The authors declare that they have no conflict of interest.

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