Original article

Absence of Simian virus 40 DNA in salivary glands among Sudanese patients with Pleomorphic adenoma

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DOI: 10.5455/jrmds.20164217

ABSTRACT

Background: Pleomorphic adenoma is a common benign salivary gland neoplasm characterized by neoplastic proliferation of parenchymatous glandular cells along with myoepithelial components, having a malignant potentiality is the commonest benign tumor to arise in the minor salivary gland. Simian virus 40 (SV40), is a highly oncogenic DNA tumor virus (polyomaviridae) which was recently found to be associated with various human tumors including pleomorphic adenoma. The virus is a monkey virus which was believed to be transmitted to humans only under exceptional situations.

Objective: The aim of this study was to evaluate the presence of SV40 DNA among Sudanese patient with pleomorphic adenoma of the salivary glands.

Material and Method: This is a case control study was conducted at Khartoum state, during period 2006-2012. Thirty study samples represents as cases that had salivary gland pleomorphic adenoma and ten normal salivary gland tissues represent as controls. Qualitative polymerase chain reaction (PCR) was used to evaluate the presence of SV40 DNA.

Results: In this study, human pleomorphic adenoma (PA) of salivary gland specimens and normal salivary gland tissues used as control were analyzed by qualitative polymerase chain reaction (PCR) for SV40. All sample (case and control) Tissue specimens were SV40 negative.

Conclusion: The findings of this study showed the absence of SV40 DNA in salivary and did not indicate any role among patients with PA.

Keywords: SV40; Salivary gland; Sudanese patients with pleomorphic adenoma

INTRODUCTION

Pleomorphic adenoma is the most common type of all salivary gland tumors, involving more frequently the parotid gland. In most series, it represents 45–75% of all salivary gland neoplasms [1]. Pleomorphic adenoma is a benign salivary gland tumor that exhibits wide cytomorphologic and architectural diversity [2]. Simian virus 40 (SV40) is a monkey virus that was introduced in the human population by contaminated polio vaccines, produced inSV40-infected monkey cells. However, the presence of this viral agent in humans, before the introduction of SV40-contaminated vaccines, cannot be discarded [3]. The molecular biology of SV40 has led to seminal discoveries in the fields of transcription, DNA replication, and oncogenic transformation [4]. Simian virus 40 (SV40), is a highly oncogenic DNA tumor virus (polyomaviridae) which was recently found to be associated with various human tumors. SV40 consists of an unenveloped icosahedral virion with a closed circular dsDNA genome [5]. The possibility that SV40 might cause tumours in humans thus became a subject of scientific and public interest and scrutiny. However, largely due to a lack of significant epidemiological evidence, interest in assessing SV40's potential carcinogenic role in humans diminished [6]. There are many hypothesis about SV40 that might cause cancer in humans has been a particularly controversial area of research. Several methods have detected SV40 in a variety of

human cancers, although how reliable these detection methods are, and whether SV40 has any role in causing these tumors, remains unclear [7]. It has been reported that mesothelioma, brain tumors, osteosarcoma and non-Hodgkin lymphoma (NHL) contain SV40 DNA sequences and that SV40 infection introduced into humans by the vaccine probably contributed to the development of these cancers [8]. These data prompted us to investigate association between SV40 and pleomorphic adenoma of the salivary glands among Sudanese patients.

MATERIAL AND METHOD

This is an analytical descriptive case control study conducted at Oral Pathology laboratory at the faculty of dentistry university of Khartoumand the Research Laboratory of Alneelin University (Khartoum-Sudan), including thirty patients pleomorphic adenoma of the salivary glands and ten normal salivary gland tissues represented the controls. Most cases analyzed in the study were distributed within the age group from 21 to 30 years.

Inclusion criteria: Specimens obtained from Sudanese patient. Confirmed salivary gland pleomorphic adenoma diagnosed by two different pathologists. Tumors from both minor and major salivary glands.
Exclusion criteria: Recurrent PA was excluded from the study.

Data Collection: The study group used data collection sheet to collect information about age, sex, description of the lesion, radiographic appearance and differential diagnosis from the patients attending to oral pathology laboratory at the faculty of dentistry university of Khartoumand the Research Laboratory of Alneelin University,(Khartoum-Sudan).

Method

Tissues were fixed in 10% formalin, processed and paraffin embedded. Specimens of pleomorphic adenoma at the stated period were 30 specimens of patients who had representing the study cases. In addition10 normal salivary gland tissues, represented the controls.

Viral DNA isolation: Viral DNA was isolated from the samples using the (innuPREP DNAmini kit, trade name: Chemicon, Millipore). Manufacturer's protocol

Viral DNA amplification: The isolated viral DNA was subjected to amplification by the polymerase chain reaction (PCR), primers designed to amplify the SV40 Tag N-terminal sequences were used (sv.For2.rev and svFor2pyv rev). The specificity of these primers had been previously confirmed by the protocol used by Martenilliet, al [(9)]. For standardizing the amplification control, additional human primers were used to exclude the chance of negative amplification due to PCR condition. Each sample was investigated twice simultaneously in a separate room to avoid contamination and false positive results.

RESULT

In this study and 30 pleomorphic adenomas were regarded as the cases and 10 normal salivary gland tissues were utilized as control. Most cases analyzed in the study were distributed within the age group from 21 to 30 years. (Table 1). The study revealed slight female predominance with a ratio of 1:1.3. (Table 2). The pleomorphic adenoma cases were diagnosed in the histopathology laboratory in the Faculty of dentistry and confirmed by 2 different pathologists (Fig 1). Twenty-two of the PA cases were found in the minor salivary glands and 8 cases in major salivary glands. The findings of the present study show there was no SV40 DNA in salivary gland pleomorphic adenoma as well as normal salivary gland tissue specimens (Table III). All samples investigated in this study using PCR method.

Table 1: Distribution of age in pleomorphic adenoma samples

Age group	Number of patients
10-20	3
21-30	16
31-40	11

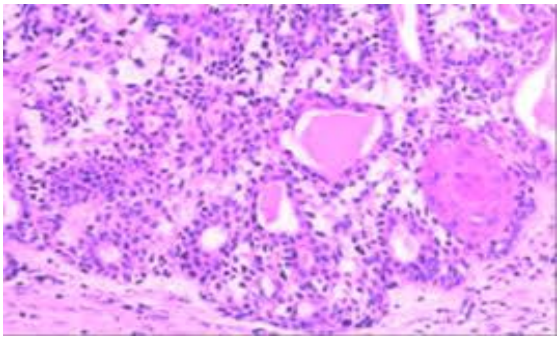
Table 2: Distribution of gender in pleomorhpic adenoma samples

Gender	Number of patients
Male	13
Female	17

Table 3: PCR results of PA samples

No.	Site	No of Cases/ %	Sex F/M	Age (mean/range)	PCR Result
1	Palate	17 / 57%	6 / 11	(23) 38-18	Negative
2	Parotid	6 / 20%	3 / 3	(30.5)40-21	Negative
3	Buccal	5 /16%	3 / 2	(25) 33-17	Negative
4	Submandibular	2 / 7%	1 / 1	(32.5) 34-31	Negative

Figure 1: Histopathological pictures of PA



DISCUSSION

Natural infection by SV40 in humans was considered a rare event, restricted to people living in contact with monkeys, the natural hosts of the virus, such as inhabitants of Indian villages located close to the jungle, and persons attending to monkeys in zoos and animal facilities [10]. Several methods have detected SV40 in a variety of human cancers, although how reliable these detection methods are, and whether SV40 has any role in causing these tumors, remains unclear [7]. In the present study the result did not indicate any finding of SV40 among patients with pleomorphic adenomas well as normal salivary gland tissue specimens. This finding agree with study conducted to evaluate the presence of SV40 and 2 human polyomaviruses BK virus (BKV) and JC virus (JCV) in a large sample of SCCs of the oral cavity and found that prevalence of SV40, BKV, and JCV in oral SCC was negligible. Matched-pair case-control analysis indicated that prevalence among the controls did not significantly differ with respect to analyzed cases [11]. Also our finding which disagreement with some findings suggests that SV40 may play a role in the onset/progression of the PA, which is a benign neoplasm [9,12-13]. Also our result found that 22 (73%) of the PA cases were found in the minor salivary glands and 8 (27%) cases in major salivary glands. This finding supported by Vicente et al. in 2008, most studies of salivary neoplasms include both the major and the minor salivary glands, and few articles focus only on minor salivary gland tumors [14]. Also another study done by Vuhahula in 2004, to outline the clinic-pathological features of salivary gland tumors in Uganda and concluded that tumors originating in the minor salivary glands are uncommon neoplasms of upper aero digestive tract [15]. Finally our negative finding of this result supported by hypothesis that more important factors than technical issues in causing differences between studies is that the prevalence of SV40 associated neoplasm may vary among geographic regions in relation with differences in exposure and /or ethnic susceptibility to SV40 infection [16]. The finding of current result as many genetics study when conducted among Sudanese peoples found differ than conducted in other nationality [17-18], this may be due to patterns and the evolutionary origins of genetic diversity present in African populations, as well as their implications for the mapping of complex traits, including disease susceptibility [19]. Finally the absence of SV40 DNA in the samples analyzed in the current study might be attributed to the difference in geographical distribution, racial and\or ethnic susceptibility to SV40 infection, or probably due to the fact that the Sudanese population were not subjected to the contaminated polio vaccine.

There were some of limitations in our study that might have an effect on the result obtained. The study was undertaken in small study samples, and more sophisticated molecular techniques for detection of SV40 had to be used such as quantitative PCR and immunohistochemistry.

CONCLUSION

Our study showed, there was no SV40 DNA in salivary gland pleomorphic adenoma as well as normal salivary gland tissue specimens and the results did not indicate any role for SV40 in the etiology of oral PA.

Conflict of Interest

The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENTS

Special thanks to the Staff of Oral Pathology Laboratory at the Faculty of Dentistry, University of Khartoum and the Research Laboratory of Al neelin University.

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Date of Submission: 08/03/2016
Date of Acceptance: 09/06/2016