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Original Article

Predictive Value of Serum p53 Antibodies in Oral Squamous Cell Carcinoma

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ABSTRACT

Background: Oral cancer represents a remarkable component of global cancer burden with marked mortality and morbidity. This study is designed to determine the predictive value of serum p53 antibodies in cases of oral squamous cell carcinoma (OSCC) and among healthy individuals.

Materials and Methods: A comparative cross-sectional study was conducted between April, 2016 to March, 2017; on serum samples of 120 subjects comprising of 60 OSCC cases and 60 healthy individuals, collected from oral and maxillofacial surgical units of various institutes. Serum anti-p53 Ab concentrations were determined by enzyme-linked immunosorbant assay (ELISA) at the department of Pathology (Microbiology section), Peshawar Medical College (PMC). Data was recorded, evaluated and analysed by SPSS version 20. Sensitivity, specificity, positive and negative predictive value, likelihood ratios, accuracy, misclassification rate, diagnostic odds ratio were calculated. Receiver operating characteristic (ROC) curve was plotted and area under the curve (AUC) was calculated for measuring the diagnostic accuracy.

Results: Among the study participants, statistically significant difference was observed for serum p53 Ab levels and status. *The ROC curve analysis (AUC=0.852) suggested that the p53 seropositivity can significantly* discriminate between the cases of OSCC and healthy individuals.

Conclusion: The present study showed that serum p53 antibodies could be a useful test due to its high sensitivity for p53 seropositivity among subjects of OSCC and healthy individuals.

Key words: Oral squamous cell carcinoma, Tumour suppressor protein p53, Antibodies, ELISA.

Introduction

Globally, cancer of lip and oral cavity represents one of the frequently occurring human malignancy with significant mortality and morbidity.[1] In Pakistan cancer statistics, it represents the second most common malignant tumour with raised incidence, mortality and prevalence.[2,3] Oral squamous cell carcinoma is the most usually occurring histopathological variant of carcinoma of lip and oral cavity.[1-3]

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OSCC is usually marked by low survival rates due to late stage diagnosis .[4] Thus improving the early diagnosis and prognosis of OSCC, investigators are persistently inquiring for a biomarkers that can help in the early stage and appropriate detection of OSCC alone or in addition to other methods like conventional oral examination (COE) and histopathological evaluation.[5] The development of oral cancer occurs as a result of multistep genetic events that leads to malignant conversion of normal oral mucosa.[6] The inactivation of tumour suppressor genes (TSGs) represents one of the key event in the development of oral malignancy.[7] p53 gene is the most immensely explored gene among the TSGs, linked with OSCC.[8] p53 gene is described to be positioned at the short arm of chromosome seventeen (17) and its

outcome protein p53 has a molecular mass of 53 kilo Dalton (kD). In cancer biology, p53 role is worth mentioning as in the research literature it has been titled as the "guardian of genome". The tumour suppressor functions of p53 are achieved via cell cycle arrest, repair of DNA, senescence and apoptosis.[6-8]
Epidemiological studies have marked the alterations in p53 gene, leading to accumulation of p53 protein in the tissue samples of oral cancerous lesions, with resultant induction of p53 auto antibodies in circulation as a part of humoral immune response.[9]
There is dearth of data on serum p53 antibodies in oral cancer patients in Pakistan.
In the present study, serum p53 antibody status was evaluated among cases of OSCC and healthy individuals to inquire into its predictive role.

Materials and Methods

The present study was conducted by adopting a non-probability; purposive sampling technique. The study was carried out after approval from institutional review board (IRB) and permissions from the head/incharge oral and maxillofacial surgical units of Peshawar Dental College (PDC), Khyber College of Dentistry (KCD) & Pakistan Institute of Medical Sciences (PIMS). The study was performed on the serum samples of 120 subjects comprising of 60 cases of OSCC (Group A) and 60 healthy individuals (Group B). The sample size of 60 in each of the study group was calculated in Software for Statistics and Data Science (STATA). Proforma was employed as a data collection tool after taking written informed consent from the patients or their attendants by explaining the objectives of the study and possible outcomes. A detailed history of the study participants was recorded on a structured proforma. Beside histopathologically diagnosed cases of OSCC the inclusion criteria comprised of cases who had not yet received any treatment for oral malignancy. While beside those individuals who failed to provide informed consent due to lack of interest, the subjects with co-existing medical illness like liver cirrhosis, acute and chronic pancreatitis and diabetes were excluded from the study.[10]The healthy individuals were those who consent for the study participant and visited the participating centres for dental treatments. 5 ml of venous blood was collected from all the patients under aseptic conditions, and stored in a disposable and non pyrogenic vaccutainer (i.e., serum separator tube with yellow colored stopper for p53 Ab analysis). The blood was centrifuged, followed by collection of serum and placed in Eppendorf tube and stored at -20°C till analysed for serum p53 antibodies via ELISA by strictly following the manufacturers instruction for each kit.[11] The concentration of p53 antibodies was measured in pg/mL using an automated microplate reader (Heales-MB 580, China) set at 450nm. The observed concentration of p53 Ab was marked as positive (401pg/mL and above) and negative (0-400pg/mL). For sero-positive test result the titre was further graded as mediumly raised (401-800pg/mL), highly raised (801-1400pg/mL) and very highly raised (>1400pg/mL).
The data obtained were analyzed by using SPSS version 20. Results were given as mean and standard deviation (SD) for continuous variables (eg., age, serum p53 antibodies concentration). Difference between the groups with categorical values were analyzed for statistical significance using Chi sqauare and Fisher's exact test, where appropriate. A probability value of less than and equal to 0.05 (i.e., $p \leq 0.05$) was considered statistically significant. Predictive value was calculated from sensitivity, specificity, positive and negative predictive value, likelihood ratios, accuracy, misclassification rate, diagnostic odds ratio by 2x2 contingency table in which the disease condition and non diseased is marked versus "positive test" and "negative test". The reference diseased states were OSCC diagnosed on biopsy and non diseased subjects were, healthy individuals. Receiver operating characteristic curve was plotted and area under the curve was calculated for measuring the diagnostic accuracy.

Results

Results of the present study are summarized in the tables (Table-1&2) and figure-1; given below with essential description.
The recorded mean age of cases of OSCC and healthy individual was 54.5(SD-14.4) and 50(SD-11.83) years, respectively with male to female ratio of 2:1.75 in each group [Table-1].
T test revealed that the difference between the mean p53 antibodies among the cases of OSCC and healthy individuals was statistically significant. Circulating serum p53 antibodies were detected in 59 (98.3%) out of 60 cases of OSCC. Among healthy individuals the serum p53 Ab concentration was above the cut off level in 33.3% [N=20/60] subjects. Also highly significant difference was observed among the study participants for the serum p53 Ab status and concentration [Table1].

Table-1: Description of age, gender, p53 antibody levels and status of the study subjects

Study variables	Study groups		p-value*
	Healthy individuals	OSCC	
Age and Gender			
Minimum age in years	28	20	>0.05[a]
Maximum age in years	73	95	
Mean±SD	50±11.83	55±14.43	
Male	32(53.3%)	32(53.3%)	1.0[b]
Female	28(46.7%)	28(46.7%)	
M:F	2:1.75	2.1:1.75	
Values of serum p53 Ab levels (pg/mL)			
Minimum	149	392	<0.01[a]
Maximum	596	4249	
Mean±SD	340.35±119.9	1211.12±795.8	
Categorical distribution of serum p53 Ab levels (pg/mL)			
0-400	40(66.7%)	1(1.7%)	<0.01[b]
401-800	20(33.3%)	20(33.3%)	
801-1400	-	23(38.3%)	
>1400	-	16(26.7%)	
Serum p53 Ab status			
Seronegative	40(66.7%)	1(1.7%)	<0.01[c]
Seropositive	20(33.3%)	59(98.3%)	

*a=t-test; b= Pearson's Chi square test; c= Fisher's exact test

Sensitivity of 98% and specificity of 66% was recorded for assessing the predictive value of serump53 antibodies in relation to OSCC. Among healthy individuals the false positive were 20 and among OSCC cases the false negative comprise of only one case (Table-2)

Table-2: Calculating the predictive value of serum p53 Ab status in OSCC cases

2x2 table for calculating the predictive value of serum p53 Ab status			
Serum p53 Ab status	**OSCC**	**Healthy individuals**	**Total**
Test Positive	59 (TP)	20 (FP)	79
Test Negative	1 (FN)	40 (TN)	41
Total	60	60	120

Statistics for assessing the predictive value of serum p53 Ab status in OSCC cases			
Sr.#	**Statistics**	**Formula**	**Value**
1.	Sensitivity /True positive rate	TP/[TP+FN]	0.98x100=98%
2.	Specificity	TN/[FP+TN]	0.66x100=66%
3.	Positive predictive value (PPV)/Precision	TP/[TP+FP]	0.74x100=74%
4.	Negative predictive value (NPV)	TN/[TN+FN]	0.97x100=97%
5.	Likelihood ratio of positive result (LR+)	Sensitivity/[1-Specificity]	2.8
6.	Likelihood ratio of negative result (LR-)	[1-Sensitivity]/Specificity	0.025
7.	Accuracy	[TP+TN]/[TP+TN+FP+FN]	0.82x100=82%
8.	Misclassification rate	[FP+FN]/[TP+TN+FP+FN]	0.175
9.	Diagnostic odd's ratio	[TPR(1-FPR)]/[FPR(1-TPR)]	94;p<0.0001;95%CI 15.2 to 914.89

***FPR**=False positive rate=**1-Specificity=FP/[TN+FP];TPR**=True positive rate=**TP/[TP+FN]**

The ROC curve is a graphical plot, constructed with sensitivity on y axis and specificity on x axis. The graph shows relation between the true positive and false positive rate. The diagonal line (green in color) making 45°angle, represents the perfect chance. The area under the diagonal is 0.5 (half of the area of graph) is null hypothesis area. The closer the curve comes to the 45-degree diagonal of the ROC space, the less accurate the test. Closer the graph to the top and left hand border, the more accurate the test. AUC is a global measure of a diagnostic accuracy for general assessment of the test. If its value is <0.5 it shows it is not a useful test, if it is 0.5-0.6, it reveal bad diagnostic accuracy while 0.6-0.7 indicates sufficient diagnostic accuracy, 0.7-0.8 good, 0.8-0.9 indicated very good diagnostic accuracy and 0.9-1 indicates excellent or perfect diagnostic accuracy. In the present study p value is less than 0.05-AUC value is statistically different from 0.5, and indicates that the test does have the ability to distinguish between two groups.

The ROC curve analysis with AUC value revealed [0.852, p<0.01] that test has very excellent diagnostic accuracy, suggesting that p53 seropositivity can significantly discriminate between healthy individuals and cases of OSCC (Figure-1).

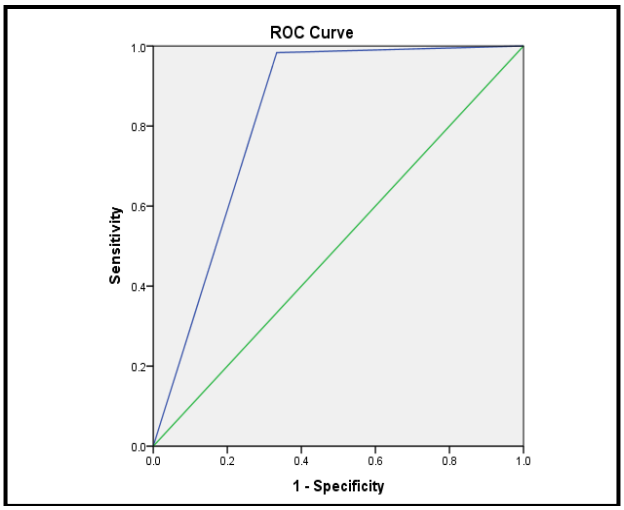

Figure -1
Receiver operating characteristic (ROC) curve analysis for serum p53 antibodies based on ELISA data in discriminating OSCC cases from healthy controls .Area under the curve 0.852,p<0.01 at 95% CI(0.746-0.904).

Discussion

Oral squamous cell carcinoma (OSCC) is the frequently occurring oral epithelial malignancy .[12]There is always a perpetual search for a biomarker that can assist in the timely prediction of detection of OSCC and thus improving its prognosis. In the present study the clinical importance of serum p53 antibody status was evaluated among cases of OSCC and healthy individuals to unfold its probable role in the timely detection of oral epithelial malignancy.

In the present study, the observed age range and mean age of the cases of OSCC is in obedience to national and internal studies.[12-14]The occurrence of most common malignancies increases with age. Age is marked as a important prognostic factor in cancer patients.[12]Age has been considered as a risk marker for the development of OSCC.[13,14]

The present study observed that OSCC occurred more commonly in males than females. These findings are comparable with other reported studies. [12-15]

In the present study serum p53 antibodies levels and status were significantly different among cases of OSCC and healthy individuals (Table-1).

The present study observed that among OSCC cases, only a single false negative sample was noted (Table-1 & 2). This observation of the present study is contrary to the noted frequency of detection of serum p53 autoantibodies as reported by Friedrich et al., (N=31/39)[16]; Warnakulasuriya et al., (N=7/26)[17], Sainger et al., (N=30/161)[18], Yamazaki et al., (N=18/113)[19], Porrini et al., (N=22/72).[20] The possible explanation may be that serum p53 Antibodies are mostly related to the type of underlying mutation related to p53.[21]

The present study revealed that among the healthy individuals, 33% (N=20/60) were false positive (Table-1 & 2). The observed frequency of seropositivity of p53 antibodies among healthy controls ranges from 0-24.2% .[16,18,20,21] This difference of the results are most probably due to demographic variation of the population under study.

However, all the healthy individuals (N=20/60) of our study, who expressed serum p53 auto-antibodies level in positive values/range but mildly high levels (i.e., 401-800 pg/mL) and none of them expressed serum p53 Ab levels in range from 801 to 1400 pg/mL or above (Table-1).

While observing the predictive value of serum p53 antibodies in diagnosis of OSCC, the present study noted that the test had a good sensitivity (98%) and was capable of diagnosing 59 out of 60 cases of OSCC but with a low specificity (66%) and was found to be ineffective in detecting disease free subjects. In other words the test was able to detect 98% of the true diseased cases while only 2% false negative were not recognized. The specificity of 66% showed that the test detected 66% subjects of healthy individuals but 34% false positive subjects were not recognized. These observations are contrary to findings noted by Friedrich et al., (Sensitivity 21.6%; Specificity 100%)[16], Sainger et al., (Sensitivity 23%; Specificity 89%)[18], and Porrini et al., (Sensitivity 16%; Specificity 88%)[20] who recorded low sensitivity and high specificity. In the present study, PPV of 74% showed that 26% results were false positive. NPV of 97% showed that those subjects who tested negative had 97% possibility of not having the disease. LR+ of 2.8 showed that it was not a good diagnostic test as it provided a weak evidence to rule in a diagnosis. The LR- (0.025) value was less than 0.1 thus it provided a strong evidence to rule out a diagnosis. High DOR showed that the test had better discrimination test performance. Misclassification rate (17%) showed a proportion of subjects, who were incorrectly classified by the test. The ROC curve [0.852 at 95% CI (0.746-0.904); p=<0.01] showed very good diagnostic accuracy of serum p53 Ab status in detection of OSCC. This observation of our study is in accordance with findings by Sainger et al., who reported AUC value of 0.796 (p=0.001) and reported that test is significantly good to discriminate between the healthy subjects and OSCC cases .[18]

The present study employed several measures [22,23] for determination of the predictive value of the p53 seropositivity among the study individuals. Beside sensitivity, specificity, positive predictive value & negative predictive value, other parameters such as likelihood ratio of positive result, likelihood ratio of negative result, accuracy, diagnostic Odd's ratio, misclassification rate and AUC analysis were used for the first time all together in the research related to serum p53 Ab among cases of OSCC and healthy individuals.

Conclusion

The present study concludes that serum p53 autoantibodies detection is the simple, rapid and non invasive method which has the ability to discriminate between OSCC cases and healthy individuals.

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Disclaimer

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Conflict of interest: None.

Ethical Approval: The ethical assent of the study was taken from Institutional review board (IRB) of Prime Foundation Pakistan (IRB Approval No. PMC/IRB/2015-003).

References

1. Nocini R, Lippi G, Mattiuzzi C. Biological and epidemiologic updates on lip and oral cavity cancers. Ann Cancer Epidemiol. 2020;4:1-6.
2. Saira, Ahmed R, Malik S, Fiaz Khan M, Khattak M. Epidemiological and clinical correlates of oral squamous cell carcinoma in patients from north-west Pakistan. J Pak Med Assoc. 2019;69:1074-78.
3. Mahmood S, Faraz R, Yousaf A, Quader A, Asif H, Atif A et al. Collective cancer registry report December 1994 till December 2018, of ShaukatKhanum memorial cancer hospital and research centre (SKMCH&RC) [Internet]. Lahore: ShaukatKhanum memorial cancer hospital and research centre (SKMCH&RC); 2019. Available from: https://shaukatkhanum.org.pk/wp-content/uploads/2019/07/ccrr_1994_2018.pdf
4. Rodriguez-Archilla A, Carrion-Ruiz M. Usefulness of salivary biomarkers in oral precancer and cancer. International Dental & Medical Journal of Advanced Research. 2018;4:1-6.
5. Jain A. Molecular pathogenesis of oral squamous cell carcinoma. In Squamous Cell Carcinoma-Hallmark and Treatment Modalities. 2019. IntechOpen. doi:10.5772/intechopen.85650.
6. Wang L, Wu C, Rajasekaran N, Shin Y. Loss of Tumor Suppressor Gene Function in Human Cancer: An Overview. Cell Physiol and Biochem. 2018;51(6):2647-93. doi:10.1159/000495956.
7. Ragos V, Mastronikolis N, Tsiambas E, Baliou E, Mastronikolis S, Tsoukalas N et al. p53 mutations in oral cavity carcinoma. J BUON. 2018;23:1569-72.
8. Efeyan A, Serrano M. p53: guardian of the genome and policeman of the oncogenes. Cell cycle. 2007;6:1006-10. doi:10.4161/cc.6.9.4211.
9. Yang ZC, Ling L, Xu ZW, Sui XD, Feng S, Zhang J. Are p53 Antibodies a Diagnostic Indicator for Patients with Oral Squamous Cell Carcinoma? Systematic Review and Meta-Analysis. Asian Pac Cancer Prev. 2016;17:109-15. doi:10.7314/apjcp.2016.17.1.109.
10. Saleh J, Mathias M. P53 autoantibodies in the sera of patients with various types of malignancies. Arab J Pharm Sci. 2003;2(5):63-71.
11. Elabscience. Human TP53 (Tumor Protein p53) ELISA Kit Manual. WuHan, People's Republic of China: Elabscience Biotechnology Co., Ltd.; 2016.
12. Mahmood N, Muhammad H, Ahmed A, Jamal Q, Saqib M, Khan A. Impact of age at diagnosis on clinicopathological outcomes of oral squamous cell carcinoma patients. Pak J Med Sci. 2018;34:595-99.
13. Alves AM, Correa MB, Silva KD, Araújo LM, Vasconcelos AC, Gomes AP et al. Demographic and clinical profile of oral squamous cell carcinoma from a service-based population. Braz Dent J.2017;28:301-6. doi:10.1590/0103-6440201601257.
14. Zini A, Czerninski R, Sgan-Cohen HD. Oral cancer over four decades: epidemiology, trends, histology, and survival by anatomical sites. J Oral Pathol Med. 2010;39:299-305. doi:10.1111/j.1600-0714.2009.00845.x.
15. Sahaf R, Naseem N, Anjum R, Nagi AH, Path FR. A study of 89 cases of oral squamous cell carcinoma presenting at Teaching Hospitals of Lahore, Pakistan. J Pak Dent Assoc. 2017;26:26-31. doi:10.25301/jpda.261.26.
16. Friedrich RE, Bartel-Friedrich S, Plambeck K, Bahlo M, Klapdor R. P53 auto-antibodies in the sera of patients with oral squamous cell carcinoma. Anticancer Res.1997;17:3183-84.
17. Warnakulasuriya S, Soussi T, Maher R, Johnson N, Tavassoli M. Expression of p53 in oral squamous cell carcinoma is associated with the presence of IgG and IgA p53 autoantibodies in sera and saliva of the patients. Journal Pathol. 2000;192:52-7.

18. Sainger RN, Shah MH, Desai AA, Shukla SN, Shah PM, Telang SD et al. Clinical significance of serum p53 antibodies in oral cancer. Tumori. 2006 ;92:134-9.
19. Yamazaki Y, Chiba I, Ishikawa M, Satoh C, Notani KI, Ohiro Y et al. Serum p53 antibodies as a prognostic indicator in oral squamous cell carcinoma. Odontology. 2008;96:32-37. doi:10.1007/s10266-008-0079-y.
20. Porrini R, Vercellino V, Rocchetti V, Reno F, Giorda E, Pomato E et al. Serum anti-p53 antibodies as a diagnostic tumor marker: observations in patients with malignant and premalignant oral cavity lesions. Minerva stomatol. 2010;59:233-39.
21. Soussi T. p53 Antibodies in the sera of patients with various types of cancer: a review. Cancer Research. 2000;60:1777-88.
22. Šimundić AM. Measures of diagnostic accuracy: basic definitions. EJIFCC. 2009;19(4):203-11.
23. Shaikh SA. Measures derived from a 2 x 2 table for an accuracy of a diagnostic test. J Biom Biostat. 2011;2(5):1000128. doi:10.4172/2155-6180.1000128.

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A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion