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Letter to the editor

## Cripto 1, a potential biomarker for oral squamous cell carcinoma



To,  
The Editor,

We read the article published in your esteemed journal in 2011 by Yoon H.J. et al with interest, where the authors for the first time reported expression levels of Cripto-1 (CR-1) in Oral Squamous Cell Carcinoma (OSCC) [1]. Yoon H.J. et al reported the levels of membrane bound form of the protein by immunohistochemistry (IHC) in the tumor tissue of OSCC cases. This is to mention that Cripto-1, a member of EGF-CFC protein family and an oncofetal protein is involved in a number of signalling pathways crucial for embryonic development [2]. CR-1 is expressed in a very low level or ceases to express during post embryonic life, except in cases of various cancers, where it is highly expressed as reported in literature [3–5]. It can be present in secretory or membrane bound form owing to the presence of glycoposphatidylinositol (GPI) anchor [6]. Our group evaluated the expression of circulatory absolute form of this protein in OSCC patients along with membrane bound one (see Table 1).

We analysed expression of CR-1 in 50 biopsy proven OSCC cases and 50 healthy controls. Circulatory form was estimated in the serum of cases and controls by sandwich ELISA, while membrane bound form was checked in tumor tissues by IHC. We found that the serum CR-1 levels were 2.25-fold high in cases as compared to healthy controls ( $p = 0.0001^{***}$ ). The mean CR-1 levels were 365.10 pg/mL in cases and 167.36 pg/mL in controls. CR-1 levels were increased more in cases of early stage cases i.e. 542.27 pg/mL (TNM stage I & II) as compared to late stage cases i.e. 309 pg/mL (TNM stage III & IV). Membrane bound CR-1 positivity in 80% of the OSCC cases were found upon IHC in the malignant tissue of the OSCC cases which is more than that reported by Yoon H.J. et al.

We followed-up our enrolled cases till the completion of their treatment protocol comprising of surgery and/or radiotherapy appropriate for their stage and grade with curative intent. Upon treatment completion, CR-1 levels were again checked in the serum of the cases. CR-1 levels decreased in cases with mean level of 260.18 pg/mL ( $p = 0.0002^{***}$ ), after six weeks of completion of therapy.

At the first follow up after six weeks of completion of therapy, cases were divided into three groups based on their post therapy disease status. First group was termed as NED (No Evidence of Disease) group, having no clinical and radiological evidence of the disease, second was termed as RD (Residual Disease) group, having residual disease and third group denoted as REC includes cases of recurrence. It was observed that in cases of NED group CR-1 levels decreased significantly ( $p = 0.0056^{**}$ ) with respect to their pre-treatment levels (Table 1). Although CR-1 levels also decreased in other groups (both RD and REC) but no statistical significance can be found in the decrease of its serum level. A Receiver Operating Curve (ROC) was also generated with the CR-1 levels data (Fig. 1), to check the efficiency of estimation of Serum CR-1 to diagnose OSCC. A sensitivity of 74% and specificity of 78% was observed at a cut off level of 202.5 pg/mL (median CR-1 value of the

control group). Area under the curve for ROC was 0.8 with a significance level of  $p = 0.0001^{***}$ .

A biomarker for OSCC is highly required for the diagnosis and management of the patients, as the disease has a high prevalence rate particularly in developing countries and the overall survival rate associated with the disease is abysmal. The high morbidity and mortality associated with the disease is mostly attributed to the late diagnosis and non-availability of a suitable tumor marker is one of the factors leading to this poor prognosis.

Yoon H.J. et al, in their original study, have reported CR-1 as a molecule of interest for targeted therapy but our data suggests that it can also be evaluated as a potential biomarker in OSCC. Our results, demonstrating the high levels of CR-1 in cases as compared to controls and decrease in CR-1 levels post therapeutically, suggests that levels of CR-1 can be evaluated further for its role as a biomarker in OSCC. Also, the significant CR-1 decrease in cases of NED group and not in cases of RD or REC group indicates that the levels can be a clear indicator of therapeutic response, thus making it a potential prognostic marker also. All these findings are definitive hallmarks of a potential biomarker. We have evaluated the levels only in 50 cases as a pilot study. Results of our study lead towards a new role of CR-1 in OSCC as a potential biomarker. This role of CR-1 in OSCC needs to be further evaluated in larger patient population with long term follow-up.

With great advancement in scientific technology, various omics based approaches like genomics, transcriptomics, metabolomics, proteomics etc. have been made towards identification and development of biomarkers for various cancers. An emerging approach in this direction includes liquid biopsy, which is analysis of genetic features of tumor by analysing the circulating genetic material (circulatory DNA) or cells (circulatory tumor cells) in the blood, which is proposed to be used to monitor the therapeutic response as well as to assess the metastatic status of the disease. Recently, P. Economopoulou et. al very well reviewed use of liquid biopsy to assess the disease burden in squamous cell carcinoma of head and neck region [7]. However, such assessment cannot always provide a true picture of the metastatic status of the disease. Definitive evidences of tumor like a measure of candidate biomarkers, such as CR-1, along with liquid biopsy can be of great help to predict a more realistic disease status in OSCC.

In 2017, Xianglan Zhang et. al also reported an interesting finding where a nomogram was used for predicting risk of progression of pre-malignant oral diseases to oral cancer [8]. They further proposed addition of other candidate biomarkers to their model to improve the performance of the model. The molecule of our interest, Cripto-1 can be one such candidate, which may be incorporated into the proposed nomogram to increase the predictive probability.

## Conflicts of interest

None.

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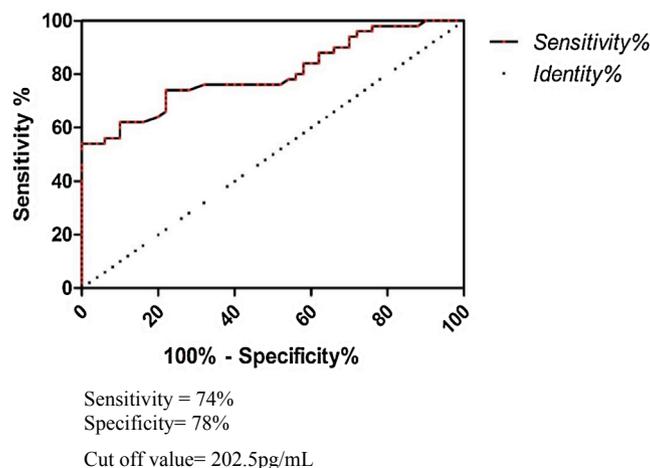
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**Table 1**

Pre and post treatment CR-1 levels in cases as per disease staging and post treatment disease status.

Characteristic	Before treatment CR-1 levels (pg/mL)	After treatment CR-1 levels (pg/mL)	p value
CR-1 levels in cases	365.10	260.18	<b>0.0001***</b>
TNM Classification			
Early stage (T1 & T2) (n = 12)	542.27	280.3	<b>0.011*</b>
Late Stage (T3 & T4) (n = 38)	309	253.8	<b>0.014*</b>
Disease Status Post Treatment			
No Evidence of Disease (n = 27)	380.4	241.9	<b>0.0056**</b>
Residual Disease (n = 14)	321.4	256	0.1629
Recurrence (n = 9)	387.12	321.53	0.08

**Fig. 1.** ROC analysis for estimated CR-1 levels; area under the curve = 0.80.**Source of financial support**

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