



ISSN: 0976-3376

Available Online at <http://www.journalajst.com>

ASIAN JOURNAL OF  
SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology  
Vol.07, Issue, 04, pp.2825-2829, April, 2016

## RESEARCH ARTICLE

### MAIN PARAMETERS AFFECTING PROMOTION OF LARYNGEAL AND ORAL CAVITY CANCERS: A STUDY FOR THE FORMULATION OF A PRATICAL RISK INDEX

<sup>1,\*</sup>Menicagli, R., <sup>2</sup>Duca, M. and <sup>3</sup>Arizzi, C.

<sup>1</sup>Roma Biomed Research "Mediglia Italy

<sup>2</sup>ENT Asst Bassini Hospital Milan Italy

<sup>3</sup>Physician Department Anat Pathol. Hospital Melegnano Italy

#### ARTICLE INFO

##### Article History:

Received 15<sup>th</sup> January, 2015

Received in revised form

28<sup>th</sup> February, 2016

Accepted 20<sup>th</sup> March, 2016

Published online 27<sup>th</sup> April, 2016

##### Key words:

Human resource management practices,  
Work outcomes,  
Perceived organizational support.

#### ABSTRACT

**Introduction and Objectives:** A recent study in laryngectomized patients, showed that other parameters, in addition to smoking and HPV, such as autoimmune diseases, sex, lifestyle, can be considered important risk factors to activate the process of carcinogenesis of the oral cavity and laryngeal. This study assigns each individual risk factor score, and identifies the theoretical threshold, beyond which we must implement a monitoring in detecting and removing those factors and symptoms that indicate a possible irreversible damage to the protective structure made from the salivary mucin the objectives of this study, to prove the validity of this index on a larger sample of laryngectomized and verify, in a first preliminary experiment if the risk factors of age, sex and smoking, with the determination of Free Radicals can vary the theoretical score, according to the concentration of malondialdehyde salivary secure indicator of cell damage

**Materials Study Method:** was administered to eighty laryngectomized a questionnaire that asked them what habits, diseases and / or symptoms, considered as a whole "risk factors", nine in total, which were present before he was diagnosed with their cancer the final data were compared statistically by the Fisher Exact Test, with those of a control population. In parallel with the experimental tests have been started to determine the saliva by dip sticks, the concentration of free radicals, which occurs in the presence of three of the major risk factors, taken individually: age, smoking and sex The calculation of the correlation between smoking, age, sex and cigarette consumption was determined by Logistic regression, establishing the cutoff values for the concentration of malondialdehyde

**Results and Discussion:** The result of questionnaire confirmed that age smoking is a significant percentage of patients with laryngeal cancer risk factor that has an autoimmune disease is higher than the figure of the Italian general population (26% vs 1%,  $p < 0.0001$ , with a test of proportions) the smoke and the presence of an autoimmune disease are not two associated risk factors ( $p = 0.1211$  by Fisher exact test). The results also indicate that the presence of an autoimmune pathology is a significant risk factor for cancers of the larynx (at least in the sample analyzed by us), having a value of 26% in laryngectomized vs 7% in the controls, ( $p = 0.0123$  by Fisher exact test). The data also suggest that there may be individual risk factors also hypogeusia and xerostomia ( $p = 0.0563$  and  $p = 0.0559$ , respectively) and that the latter could be a specific risk factor for men (27% in men laryngectomized vs 3% of the control men,  $p = 0.0260$  by Fisher exact test). Smoking  $\leq 7$  cigarettes / day, it does not involve a significant increase in MDA control, compared with the men's group, while smoking  $\geq 7$  cigarettes / day, implies a significantly increased at risk Older age  $\geq 45$  years, it has a significant effect on increasing the MDA, both in smokers, and men in the control group The increase in the concentration of salivary MDA value (greater than 3 nM / ml) in the control group women's relative to 21% of the control group for ages above 49 years. These indicate that the age, gender and smoking can substantially be reassessed score for the theoretical effect of the concentrations of salivary MDA found in smokers compared with the two control groups of men and women

**Conclusions:** It can indicate the age factor in man, three values of cut-off, obtained either from the control population; the first regards younger than 40-44 years with values of less than 3 nM MDA / ml, the second age 44-60 with concentrations of MDA  $\geq 3 \leq 5$ , and a third of persons who have passed these years

For women, you have to change the values 0/1, depending on the major or minor age of sixty years, but you have to take as a reference the average age of onset of menopause

FOR As for the other risk factors you will have to determine the salivary MDA for autoimmune diseases and in different life styles, without affecting its overall score assigned to others risk factors

Copyright © 2016 Menicagli et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## INTRODUCTION

The ASR incidence ratios in the database Globocan 2012 (IARC), highlighting that the tumors of the larynx, but especially those of the oral cavity, have the highest values where for example there are special dietary habits (betel), (Menicagli, 2014), and / or also with poor tobacco alcohol consumption as indicate in s table 1, (WHO, 2009; Veyl et al., 2015) and low presence of the HPV virus.

These data have been confirmed both by epidemiological studies (Veyl et al., 2015), both by the results of recent experimental work focused on the role that certain substances have the alteration the mucosal layer that protects these anatomic structures, (Menicagli et al., 2016; Heather et al., 2014). The results of research on the possible genes involved with their mutations in the cancer process have shown that in the process of formation of laryngeal cancer for many of them, among them the two main Tp53 CDKN2A and their activation is also due to a possible genetic predisposition. In general, this "trend", is autosomal dominant, and implies SNP mutations

\*Corresponding author: Menicagli, R.,  
Roma Biomed Research "Mediglia Italy.

and the last "Report" have identified nineteen genes, fourteen in our recent study (Nature Report, 2016), presenting similar mutations and in the various types of cancer of the oral cavity which is of larynx. These studies show that the frequency of the various gene mutations, distinct mutations by type (missense, frame-shift etc) is not always correlated with, the smoking and, the HPV status and varies, depending on the anatomic sites of appearance of tumors. The result of this detailed analysis, however, it indicates that the genes involved in most cases are the percentage of p53 and T CDKN2A, with type missense mutations. or with the mutation of a single nucleotide. Although such mutations can be inherited, (loss of the ' homozygosis , heterozygous for the healthy allele), they do not necessarily lead to cancer, because there is a need of another mutation ', Loss of heterozygosis (LOH), to delete the correct expression for example in the p53 with the cell control. Then loss in most of the involved gene mutations and found in various types of cancer, it must be in the presence of considerable environmental insults and / or pathological, because beginning the process of carcinogenesis which need in each case of the second mutation.

These considerations not only statistics should direct research, in addition to the understanding of the genetic mechanisms that lead a cell to become cancerous, and perhaps even in the most urgent terms the adoption of measures to prevent the alteration of the oral argument as are the Salivary Mucins, from the attacks of exogenous and endogenous agents. In recent articles (Menicagli *et al.*, 2016; Heather *et al.*, 2014) as already we said, epidemiological and, both experimental studies, have permission to speculated, that this fraction of the salivary proteins, can be inactivated and / or precipitated by type of polyphenolic compounds, present in more or less high concentration in spices consumer. Also other different conditions, without considering the smoke and HPV, or the age, autoimmune wrong lifestyle diseases, can then lead to an over-production of free radicals that can act on mucins from first, and then on the same cells. In a previous study (Duca *et al.*, 2015) were examined all of these risk factors, in a retrospective analysis with the administration of a medical history questionnaire to patients laryngectomees. Has been defined even theoretically a total risk index (the sum of the scores given to various each parameter), that the results obtained would be exceeded in retrospect, from about seventy percent of patients compared to fourteen of the control population. The objectives of this study, therefore, are essentially two:

- a-check the results of this first survey, expanding the audience of laryngectomees patients who administer the questionnaire.
- b-a study to link the concentration of free radicals in the saliva, three risk factors, smoking age, and sex,
- c- check whether each numerical index relating to each individual risk factor can be changed in function of the concentrations of MDA salivary through the recognition of valid values of cut-off by means of which change the theoretical risk index in a more clinically objective.

## MATERIALS AND METHODS

In a population of eighty laryngectomees, and an equal number in the questionnaire in the healthy control it was administered in table 1. It specifies eight risk factors and two symptoms

related to them. A each risk index has been assigned a numerical value indicating the potential degree of danger, damage and / or modifications that may suffer the mucosal layer of the study data were analyzed with Fisher Exact Test. It 'was then established a priori, a risk index (cut-off),  $\geq 8$ , for which it can be assumed from literature data, the examination date and the prospective symptomatology probably present, indicating the danger threshold beyond the waste as there may be a significant alteration of the mucosal layer. For semi-quantitative determination of salivary MDA was used test strips of DFI (Dream Future Innovation), and for reading the results was used the FRC 505 Analyzer in the same DFI. The fair Expressed in nM / m l in salivary MDA is Expressed in four different ranges:

$\leq 3$  mM / L NORMAL; CAUTION 3-5; HIGH 5-7; VERY HIGH  $\geq 7$

Saliva samples were collected from groups of volunteers, 25 men smokers versus 25 men and eighteen women healthy population in the morning and after two hours of taking food, and reviewed instantly, for immersion in the fluid dipstick and read them with the FRC 50 Scanner. From the study is excluded people with diabetes ,cardiovascular disease.non moderate alcohol and using antioxidant products. The group of smokers has been divided into three subgroups according to Functional the quantity of cigarettes / day smoke

Group 1  $\geq 20$  cigarettes / day; Group 2 7 / day; Group 3  $\leq 7$  cigarettes / day: "light smokers"

All fifty volunteers, and not smoking, were then divided into two groups based on age:

Group 1  $\leq 45$  years; Group 2  $\geq 45$  years.

The study results were statistically processed by the method: "logistic ordinal regression"

## RESULTS

In table 1 are reported the results concern the influence and the incidence of smoking on ASR index for oral and laryngeal cancer in the principal countries where the epidemiology shows the highest incidence for the two cancers. All data on the various tests carried out and illustrated in the above table, 4 a were statistically analyzed using "ordinal logistic regression method", see table 5. In accordance with this statistical analysis, it may be concluded that:

- smoking level 2 or 3, implies a significantly increased risk
- smoking Level 1, it does not involve a significant increase in MDA compared with the control.
- Older age  $\geq 45$  years, it has a significant effect on increasing the MDA, both in smokers, and in the men group control
- The increase of the value of the concentration of salivary MDA (greater than 3 nM / ml) women in the control group is related to 21% of the control group for age exceed

Table 1. Factors risk index with score

RISK FACTORS									
Age Year	Sex	Smoking Cigarettes /day	Alcohol	Family Cancer	Life Style	ABO Group	Autoimmune Diseases	Xerostomia	Hypogeusia
SCORE									
≥65= 1 ≤ 65=0	M=1 W=0	TOBACCO CONSUME 0 = absent 1=ex smoker 2/3=habitual smoker	0=normal 1=strong consumption	Number 0=absence 1 or 2= More presence of cancer in family	0 1 2 Diet Sex Drugs Sport	A=1 B=1 AB=0 /O =0	Diabetes = 2 Others =1	Absent =0 Present =1	Absent =0 Present = 1

Table 1. Oral and laryngeal cancer asr index and relative tobacco consumption

Oral cancer			laryngeal cancer		
Country	Asr index	Smoking	Country	Asr index	Smoking
Papua new-guinea	25.4	+	Cuba	7.6	+
Maldive	11.0	-	Hungaria	6.4	++
Shri lanka	10.3	+	Iraq	5.6	+
Bangladesh	10.1	+	Kazakistan	5.5	++
Pakistan	9.9	+	Uzbekistan	5.5	++
Hungaria	9.9	++	Moldavia	5.3	++
India	7.9	+	Romania	5.0	++
Portugal	6.8	++	Montenegro	5.0	++
Slovacchia	6.5	+	Bulgaria	4.9	++
Australia	6.3	+	Croazia	4.7	++
Kazakistan	6.3	++	Siria	4.6	+
Myanmar	6.3	+	Portugal	4.6	++
Azerbaijan	6.3	++	Turkmenistan	4.5	+
Afganistan	6.3	++	Venezuela	4.4	+
Kambogia	6.0	+	Poland	4.3	++
Italy	3.1	++	Italy	3.2	++

Legend: smoking -/+/++ proportional values index in the tobacco consumption

Table 2. Analysis of risk factor laryngectomees group versus control group

Risk factor	Laryngectomees group 80 people			control group 80 people			Fisher exact test vs/control
	Men ≥ 65 years (%)	Women ≥ 60 years (%)	Total and (%)	Men ≥ 65 years (%)	Women ≥ 60 years (%)	Total and (%)	
Age	48 (60)	5 (6)	53 (66)	28 (35)	16 (20)	44 (55)	
Smoking	50 (63)	4 (5)	54 (68)	29 (36)	6 (7.5)	35 (43.5)	0.0891
Alcohol	5 (6)	0	5 (6)	5 (6)	0	5 (6)	1.000
Cancer family	6 (7.5)	4 (5)	10 (12.5)	3 (4)	5 (6)	8 (10)	0.0781
Xerostomia	22 (27.5)	3 (4)	25 (31.5)	2 (3)	1 (1)	3 (4)	0.0412
Hypogeusia	8 (10)	3 (4)	11 (14)	1 (1)	1 (1)	2 (2)	0.0561
Life style	37 (46)	7 (9)	44 (55)	22 (27.5)	31 (39)	53 (66.5)	1.000
Abo group	18 (22.5)	6 (7.5)	24 (30)	6 (7.5)	10 (12.5)	16 (20)	0.154
Autoimmune diseases	23 (29)	3 (4)	26 (33)	1 (1)	6 (7.5)	7 (9)	
	14 (17.5)	1 (1)	15 (18.5)	0	4 (5)	4 (5)	0.0122

Table 3- laryngectomees and control group onset cut-off ≥ 8

Laryngectomees group				Control group				
	%	Women	Total %	men	%	Women	%	Total %
total	52	6	72.5	13	16	5	6	22
for-age (40)	77	4	67	11	84	4	80	
For-smoking (44)	85	4	67	11	84	4	80	
For-xerostomia (18)	34	3	50	2	15	1	20	
For-autoimmune Diseases (18)	18	3	50	4	30	3	60	
Others	7	3	50	7	5	5	100	

DISCUSSION

As mentioned in the presentation of the results given in Table 1, it can certainly not be said that there is an absolute correlation between tobacco consumption and incidence of cancer, especially those related to oral cancer. The data shown in Table 2, confirm this assertion: post surgical control in fact 66% of laryngectomees (60 men and 6 women) at diagnosis had more than sixty-five years, but in controlling this percentage was not much lower, 55% (35 men and 20 women).

We must therefore seek other parameters that cause dangerous genetic mutations, and at the same time facilitate the startup process, in terms of alterations in the mucosal layer caused by the same parameters (see table 3), as the presence of autoimmune diseases, p-value = 0.0122. The age is one of these factors: in fact, while the flow rate of the buccal glands is significantly lower in children than in adults the number of labial glands is much higher and tends to decrease with age (Sonesson, 2011). Moreover young is the lower the concentration of MUC7 making saliva more fluid properties with more lubricant.

**Table 4. Salivary mda levels in smokers and control population legend: ^ cigarettes consumption / day ; (1) ≤ 7 - (2) ≥ 7 ≤ 20 - (3) ≥ 20 /**

Smoker S- Age -	Cigarettes*	Mda Nm/MI	Men Not Smoker Age-	MDA /MI	Women Not Smokers Age	MDA Nm/MI		
1	71	2	5-7	1	54	3-5	48	≤3
2	73	3	5-7	2	78	≤3	46	≤3
3	72	1	3-5	3	77	3-5	33	≤3
4	70	2	3-5	4	55	3-5	44	≤3
5	66	1	3-5	5	55	≤3	39	≤3
6	65	3	5-7	6	52	≤3	35	≤3
7	66	1	≤3	7	70	≤3	28	≤3
8	55	3	≥7	8	53	3-5	23	≤3
9	54	3	3-5	9	59	3-5	29	≤3
10	49	1	≤3	10	46	3-5	44	≤3
11	47	2	3-5	11	55	≤3	39	≤3
12	47	1	≤3	12	50	≤3	27	≤3
13	25	2	3-5	13	44	3-5	33	≤3
14	44	3	3-5	14	27	≤3	40	≤3
15	32	1	≤3	15	32	3-5	49	≥3≤5
16	30	2	3-5	16	24	≤3	52	≥3≤5
17	27	2	≤3	17	33	≤3	54	≥3≤5
18	43	3	5-7	18	39	≤3	60	≥3≤5
19	32	3	3-5	19	38	≤3		
20	34	2	≤3	20	34	≤3		
21	36	1	≤3	21	27	≤3		
22	38	2	5-7	22	20	≤3		
23	38	1	3-5	23	18	≤3		
24	37	1	≤3	24	39	≤3		
25	20	2	3-5	25	20	≤3		

**Table 5. Statistic elaboration with “logistic regression method” results of concentration MDA (nm / l), for age and consumption of cigarettes**

Parameter	B	Hypothesis Test			Exp (B)	95%Wald Confidence Interval for Exp(B)		
		Wald Chi Square	df	Sig		Lower	Upper	
Intercept	MDA≤3/ 3-5	2.274	8.088	1	0.04	26.425	2.767	252.384
	MDA 3-5/≥5	5.287	17.043	1	0.00	537.426	27.169	10630.714
Age		0.45	4.744	1	0.029	1.004	1.004	1.089
Smokers 3 vs 0		4.986	17.448	1	0.00	146.290	14.101	1517.633
Smokers2vs 0		2.411	6.496	1	0.011	11.144	1.745	71.154
Smokers1vs 0		0.251	0.82	1	0.775	1.298	0.230	7.186

With the age, the number of the acini decreases and increases the amount of adipose and fibrous tissue and also, animal studies have revealed that the synthesis salivary proteins is reduced by 60% in old age. In human studies, (Denny, 1991) the concentration of sIgA in the labial saliva and the concentrations of mucins in low molecular weight are reduced with age. Since sIgA and mucins are important in immunological and non-immunological defense of the oral cavity, both defenses are reduced in the elderly even if healthy. Analysis of these data suggests that oral soft tissues can become a bit 'more susceptible to environmental factors due to the reduction of immunological and non-immunological defense systems. The real problem, however, arises in wanting to assign a score to the real age factor, and not arbitrarily assign that score 0 or 1, whichever is greater or less than sixty-five for men and sixty for women, as we previously done by following the directions of the epidemiological investigations IARC GLOBOCAN 2012. One solution may be suggested by a review of the results expressed in Tables 4 and 5 where the data of the salivary concentrations of MDA, relative to control groups, or non-smoking men and women, involve two important results: Older age ≥45 years, it has a significant effect on increasing the MDA, both in smokers, and men in the control group the increase in the value of the concentration of salivary MDA (greater than 3 nM / ml) in the control group women is related to 21% of the group control for ages above 49 years of age or the average value menopausal.

The Increase of MDA with age, and its secondary effect on. Mucin layer alteration may be explained and admitting the theory of Harman (Gonzalez-Freire *et al.*, 2015) or mitochondrial hypothesis expressed already in 1956. The mitochondrial dysfunction has long been considered a major cause of aging and age-related diseases. Mitochondrial free radical theory of aging postulated that somatic mitochondrial DNA mutations accumulated on the life causes excessive production of mucins reactive oxygen species that damage macromolecules and affect the function of cells and tissues. In fact, studies have shown that the oxidative maximum capacity decreases with age, while Explained Increases reactive oxygen species production. This theory has been challenged, modified and extended by many research, but remain a strong key arguments in support of it, There is evidence that an imbalance between antioxidant / oxidant occurs with age and this results in an accumulation of oxidized macromolecules and damaged and second, the accumulation of oxidative damage causes by involving the formation of a phenotype aged and degenerated. An other question concern also the score for women age Past research placed in doubt the protective role of hormones in tumor onset of mouth and larynx. it was recorded on the basis of some epidemiological data that without risk factors, such as alcohol and smoke over a certain age, (55- years about) the impact of cancer on the mouth was higher in women (Andrade, ?). Latest research but bring attention to how the endocrine microenvironment might actually be an important element in

addition to traditional other risk factors as the smoke, the HPV infection and alcohol in the training tumor process. Was proven fact (Purshotam Nainani *et al.*, 2014) that the receptors of sex hormones are given even mouth, and in larynx into the lungs, more than that in sexual organs and that these plays a key role in the expression of some of genes involved in the complex mechanism that door the process of carcinogenesis. Studies in a series of large HNC patients have shown that the estrogen levels in females play a protective role in developing cancer. as male has lesser of estrogen level, they are more predisposed to develop cancer (Zhang, 2016; Kuklinsky, 2016). Estrogen and progesterone receptors are present in oral cavity, larynx, hypopharyngel, while alcoholic patients with chronic liver disease have altered metabolism of sex hormones involving testosterone and estrogen; certain salivary gland tumors are similar to breast cancer. It is clear therefore that the conclusions of this initial research on the definition of a new risk sor for the various factors in general, and the age, sex and smoking can essentially be re-evaluated and changed in the light of recent literature data and the results obtained from the experiments for research of salivary concentrations of MDA in smokers compared with the two control groups of men and women

### Conclusion

It can indicate the age factor in man, three values of cut-off, obtained either from the control population; the first regards the younger than 40 -44 years with values of less than 3 nM MDA / ml, the second age 44-60 with concentrations of MDA  $\geq 3 \leq 5$ , and a third of persons who have passed these years. For women, you have to change the values 0/1, depending on the major or minor age of sixty years, but you have to take as a reference the average age of onset of menopause. By relating all data related to age, sex, and smoking status, the setting of the first questions of the questionnaire could be formulated by applying a grid of scores as shown below, in table 6. For the other risk factors you will have to determine the salivary MDA for autoimmune diseases and in different life styles, without affecting its overall score assigned to others risk factors. The end result will be that by filling in a questionnaire and easy detection of salivary MDA using a normal dipstick direct visual reading, you can make a real screening preventing the cancers of the oral cavity and larynx.

### REFERENCES

Andrade Jo, Associated Factors with Oral Cancer. Rev. Bras. Epidem. Dic., 18(4), 894-905

- Denny, P. C. 1991. age related changes in mucins from human whole saliva. *J Dent Res.*, Oct; 70(10):1320-7.
- Duca, M., Menicagli, R., Rancoita, P.M. 2015. Preliminary study onlaryngectomees for application of a new questionnarie for predictive screening in oral and laryngeal cancer *Frontiera ORL YearVI,N1,June-July,1-22*
- Globocan, 2012 (IARC).
- Gonzalez-Freire, M., de Cabo, R., Bernier, M., Soliott, S. J., Fabbri, E., Navas, P., Ferrucci, L. 2015. Reconsidering the role of mitochondria in aging *Gerontol A Biol Sci Med Sci*; May
- Heather, S. Davies, Paul, D. A. 2014. Pudney Pantelis Georgiades, Thomas A. Waigh, Nigel W. Hodson,Caroline E. Ridley, Ewan W. Bl Thornt ; Reorganisation of the Salivary Mucin Network by Dietary Components: Insights from Green Tea Polyphenols ;*Plose One* September 29.
- hhttp/ / www.linkiesta.it – 2013.
- Kuklinsky If sex hormones and the risk of keratinocyte cancers among women in the united states: a population-based case-control study. *Int j cancer.* 2016 mar. 3 doi: 10.1002/ijc.30072.
- Menicagli, R. and Duca, M. 2014. Possibile correlazione tra carenza dimucina nella saliva e cancro del cavo orale. Da un’analisi di particolari abitudini alimentari in paesi del subcontinente asiaticouna nuova ipotesi di lavoro *Frontiera ORL anno v numero I*
- Menicagli, R., Duca, M., Rancoita, P. M. V. 2016. Traditional Food Habits and their Possible Relationship in Diseases of the Mouth: *The Use of Paprika MR Journal*, Jan ,pp 1-7
- Nature Report 2016 28 March.
- Purshotam Nainani, Aparna Paliwal, Neelu Nagpal, and Mayank Agrawal; Sex hormones in gender-specific risk for head and neck cancer: *A review J Int Soc Prev Community Dent.* 2014 Nov; 4(Suppl 1): S1–S4
- Sonesson, M. 2011. On minor salivary gland secretion in children, adolescent and adults. *Swed Dent J Suppl.*, (215):9-64–
- Veyl, D. et al., 2015. Change in the incidence of diabetes mellitus inoral cancer patients based on long-term comparative study.*Fogorv.sz.* Mar 108(1):9-12
- WHO REPORT 2009.
- Zhang, J. Epidemiologic characteristics of oral cancer: single-center analysis of 4097 patients from the sun yat-sen university cancer center. *Chin j cancer.* 2016 mar 3; 35(1):24. doi: 10.1186/s40880-016-0078-2

\*\*\*\*\*