Quantitative Expression of Free Salivary Transcriptomes (IL8, IL1B, H3F3A) in Oral Squamous Cell Carcinoma and Oral Potentially Malignant Disorders

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INTRODUCTION

- OSCC constitutes ~90% of all oral malignancies. It usually arises from a pre-existing OPMDs that had an increased risk for malignant transformation.
- OPMDs include a variety of conditions like leukoplakia erythroplakia, erosive oral lichen planus, and oral submucous fibrosis
- The key challenge to reduce the mortality and morbidity of OSCC is to develop strategies to identify and detect OSCC when it is at very early stage, which will enable effective intervention and therapy.

- Saliva reflects hormonal, immunological, toxicological and infectious disease markers.
- Saliva sampling is noninvasive, simple, low-cost method and does not bother the patient.
- It contains numerous biomarkers used for detecting and monitoring of oral and systemic health (ma et al., 2011).
- The level of IL-6 protein was related to the severity of dysplastic changes in leukoplakia (Sharma et al., 2011).

  While IL-8 protein proved to be an analyte for early OSCC detection (Bonne and Wong, 2012).

At molecular level

- The quantity of transcriptome (mRNA, rRNA, tRNA and non-coding RNA) is changed in relation to health and disease (Hurley et al., 2012, Elliott, 2014, and Lee et al., 2011).
- There are ~ 3,000 mRNAs in cell-free saliva. Only 180 of them are common among healthy subjects (Li et al., 2004b).
- salivary transcriptomes reflects those originate in distant diseased tissues as well as transcripts that originate in salivary glands (Vlassov et al., 2012).

Previous studies

- The pioneer studies (2004) considered IL-8, SAT, IL-1B, and OAZ1 as potential biomarkers for OSCC detection (Li et al. ; Al-Reyahi).

  And specified that only salivary IL-8 mRNA had a high sensitivity and specificity.

- Later on, further studies pre-validated these biomarkers and confirm their feasibility in the discrimination of OSCC patients from control subjects (Zimmermann and Wong, 2008, Elashoff et al., 2012).

Therefore,

The quantitative expression of different salivary transcriptomes make saliva as a sensitive novel resource that could readily apply in clinical diagnosis.

Aims

The present study aims to investigate and quantify IL1B, IL-8, and H3F3A salivary transcriptome from the cell-free saliva of patients with OPMDs and OSCC patients.

Then evaluate their diagnostic value as a biomarker for OSCC detection.

Sample and methods


18 Samples
6 OSCC (stage T1)
non-treated patients
6 OMPDs (=OLP)
non-treated patients
6 Healthy individual

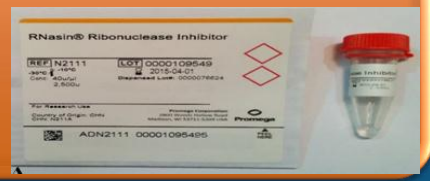
Steps :

1. **Saliva collection and RNA isolation, 2. Samples checking 3. Reverse transcription (cDNA synthesis), 4. Real-time PCR (cDNA amplification and quantification), 5. Data analysis.**

5ml saliva → 100 µl supernatant

Add RNase inhibitor.

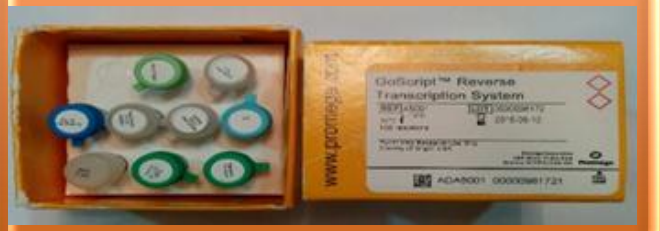
RNA extracted (cell-free saliva) using RNA extraction kit.



RNA quantification

The amount and purity measured by NanoDrop machine

cDNA produced by using cDNA kit.



Amplifed by specific primers

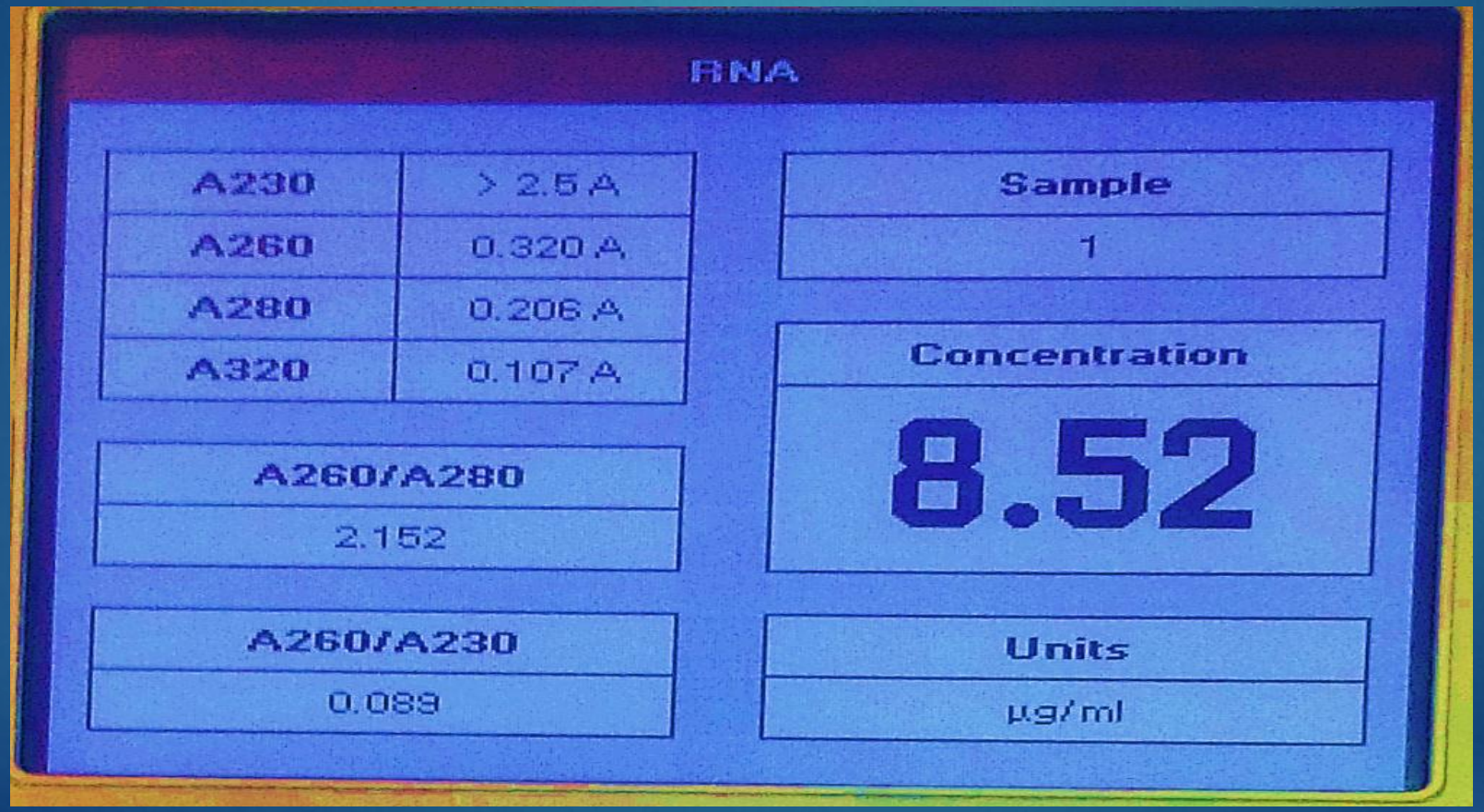
Quantified by SyberGreen reagent and qRT-PCR.

Agarose gel electrophoresis:

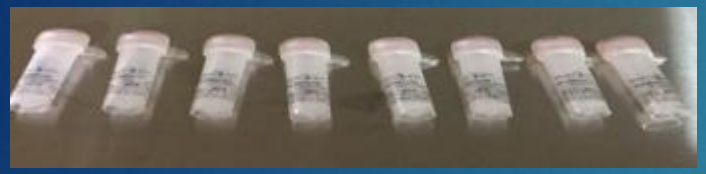
Bands of the DNA visualized and captured using gel documentation system.

Data analyzed by comparative CT (2-ΔCT) method.

RNA concentration and purity:

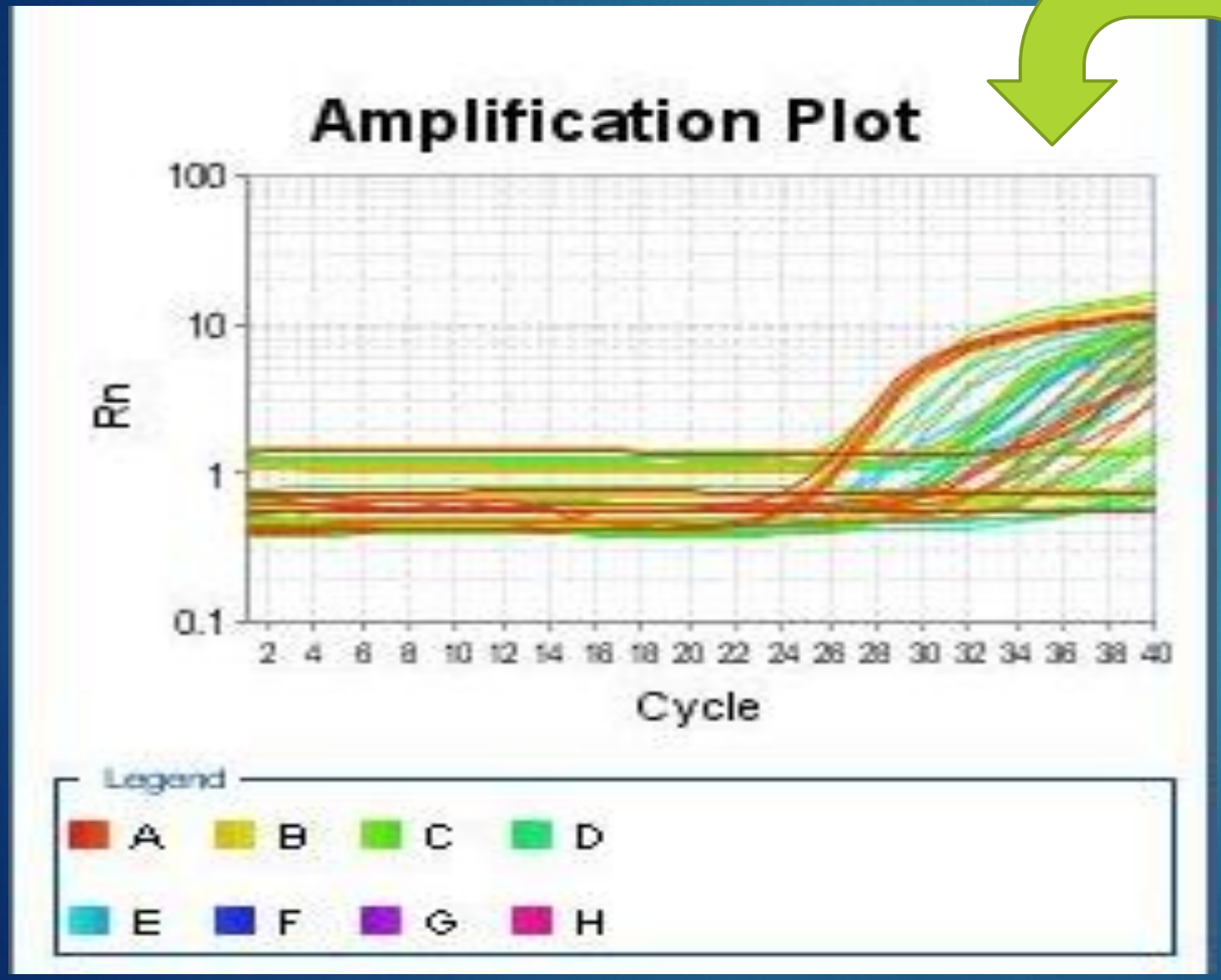


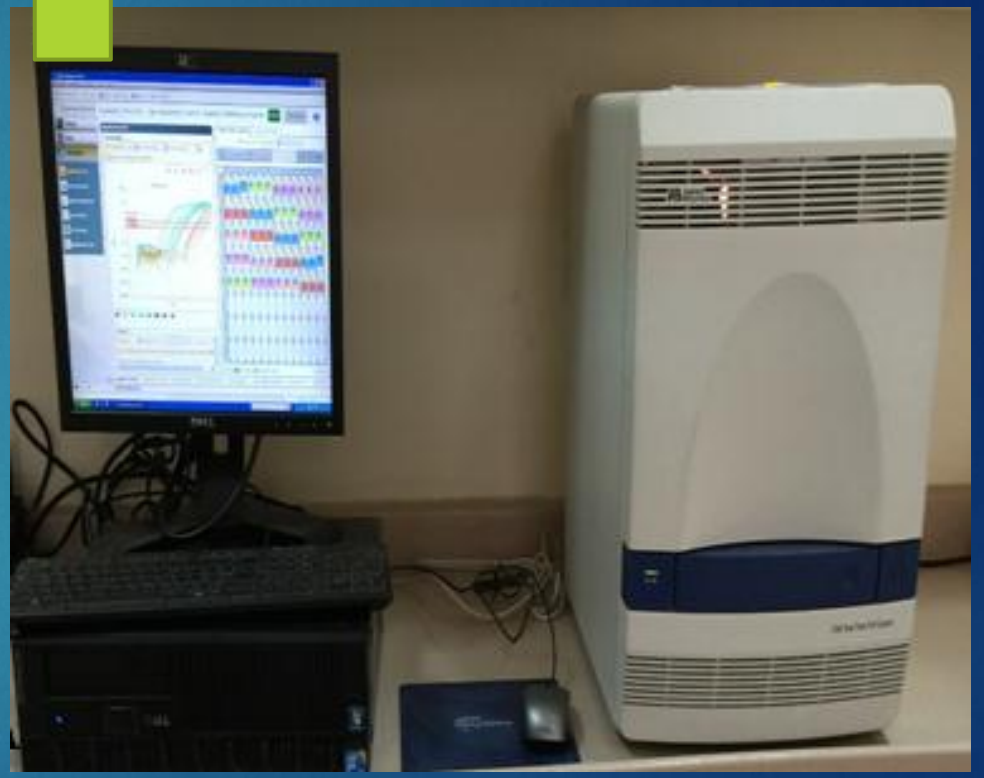
PCR primers used to amplify mRNA of *GAPDH, IL8, IL1B, H3F3A* genes



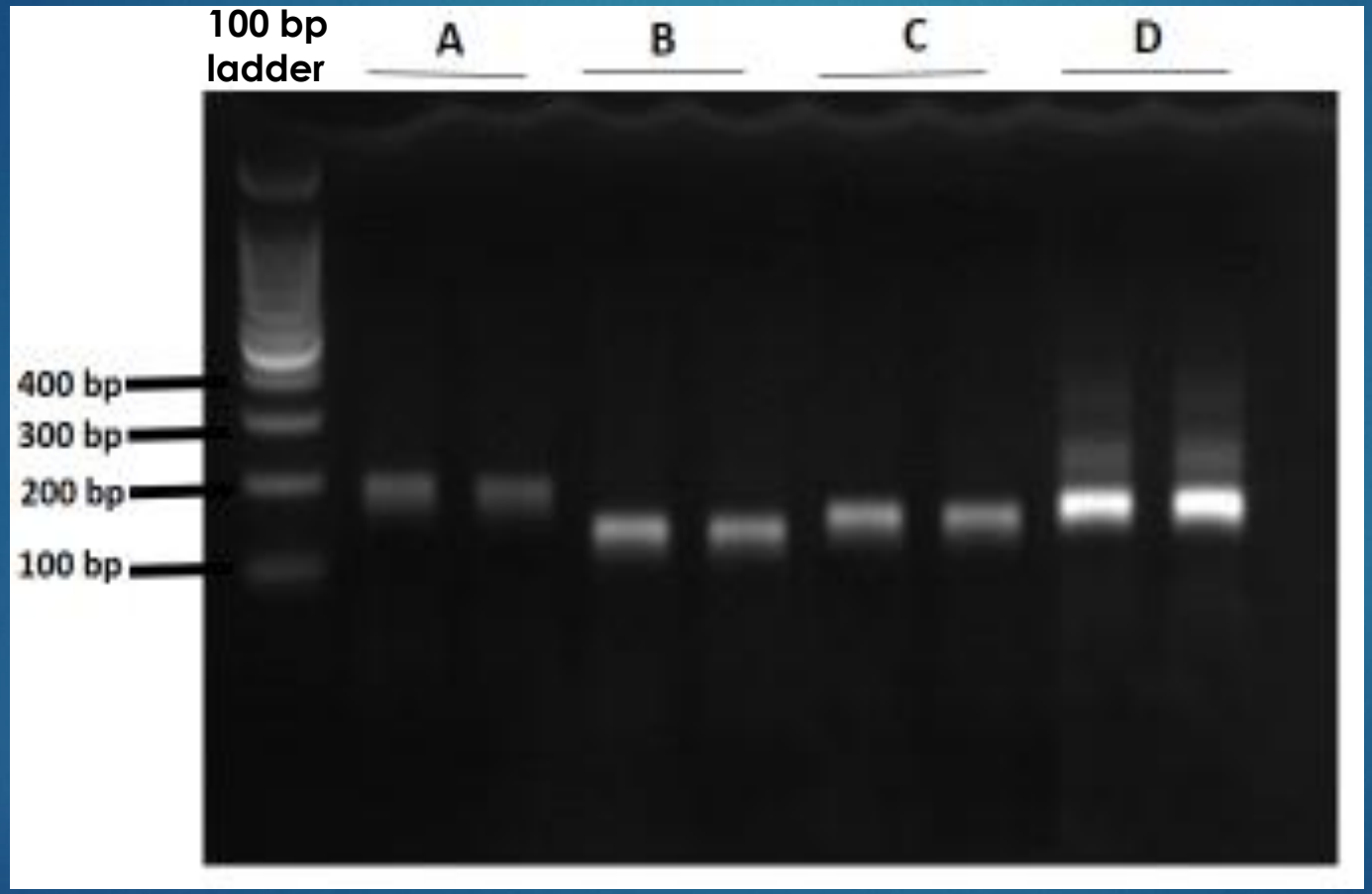
Target genes	Primer sequences (5'- 3' for forward primers) and (3'-5' for reverse primers)
GAPDH	Forward: GTCAAGGCTGAGAACGGGAA Reverse: AAATGAGCCCCAGCCTTCTC
IL8	Forward: GGTGCAGTTTTGCCAAGGAG Reverse: TTCCTTGGGGTCCAGACAGA
IL1B	Forward: CCACCTCCAGGGACAGGATA Reverse: AACACGCAGGACAGGTACAG
H3F3A	Forward: CCAGGAAGCAACTGGCTACA Reverse: CAGACGCTGGAAGGGAAGTT

with the help of the primer designing program (primer3 web-based primer design tool


Amplification Plot
100
10
1
0.1
Rn
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
Cycle
Legend
A
B
C
D
E
F
G
H



Gene product verification:



Letters A-D correspond to *IL8*, *IL1B*, *H3B3A*, and *GAPDH* genes respectively.

Gene expression and quantification:

Calculation method according to Livak and Schittgen 2001 and 2008)

Salivary transcriptome quantification in control group

	GAPDH	IL8
C1P	25.16619	28.64717
C2P	24.88572	28.23444
C3P	24.01857	28.3178
C4P	25.01857	29.24684
C5P	24.26017	28.92071
C6P	24.15162	28.64175

	Delta CT
	IL8
C1P	3.480983
C2P	3.348713
C3P	4.299229
C4P	4.228277
C5P	4.660537
C6P	4.490122

	2(-Delta CT)
	IL8
C1P	0.089561
C2P	0.098161
C3P	0.050793
C4P	0.053353
C5P	0.03954
C6P	0.044498

	Folds of difference
	IL8
C1P	0.870376
C2P	0.953946
C3P	0.493617
C4P	0.5185
C5P	0.38426
C6P	0.43244

RESULTS and Discussion:

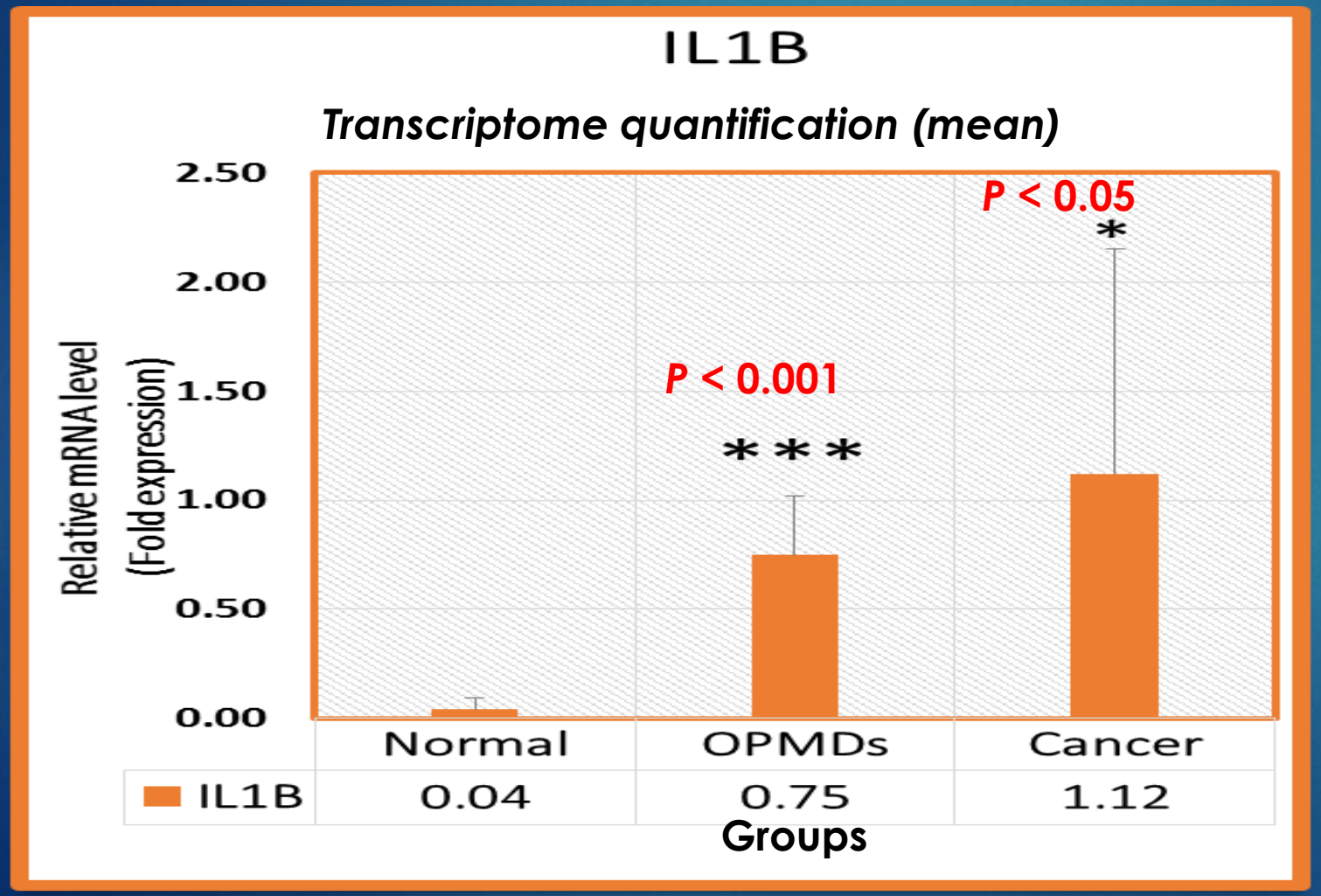
Salivary transcriptome quantification and fold change in study groups and control

Salivary transcriptomes	control	OPMD(OLP)			OSCC		
	Mean ± S.D	Mean ± S.D	folds	P-value	Mean ± S.D	Folds	P-value
IL1β	0.04 ± 0.05	0.75 ± 0.27	0.71	<0.001	1.12 ± 1.03	1.08	<0.05
IL8	0.15 ± 0.09	0.61 ± 0.24	0.46	<0.01	0.61 ± 0.20	0.46	<0.01
H3F3A	0.33 ± 0.35	0.65 ± 0.33	0.32	>0.05	0.96 ± 0.60	0.63	>0.05

The relative mean mRNA expression level of IL1B in OPMDs and OSCC

significant increase in fold expression.

OSCC=1.08 fold, OLP=0.71 fold & healthy= 0.04 fold.



ILIB is a multifunctional cytokine

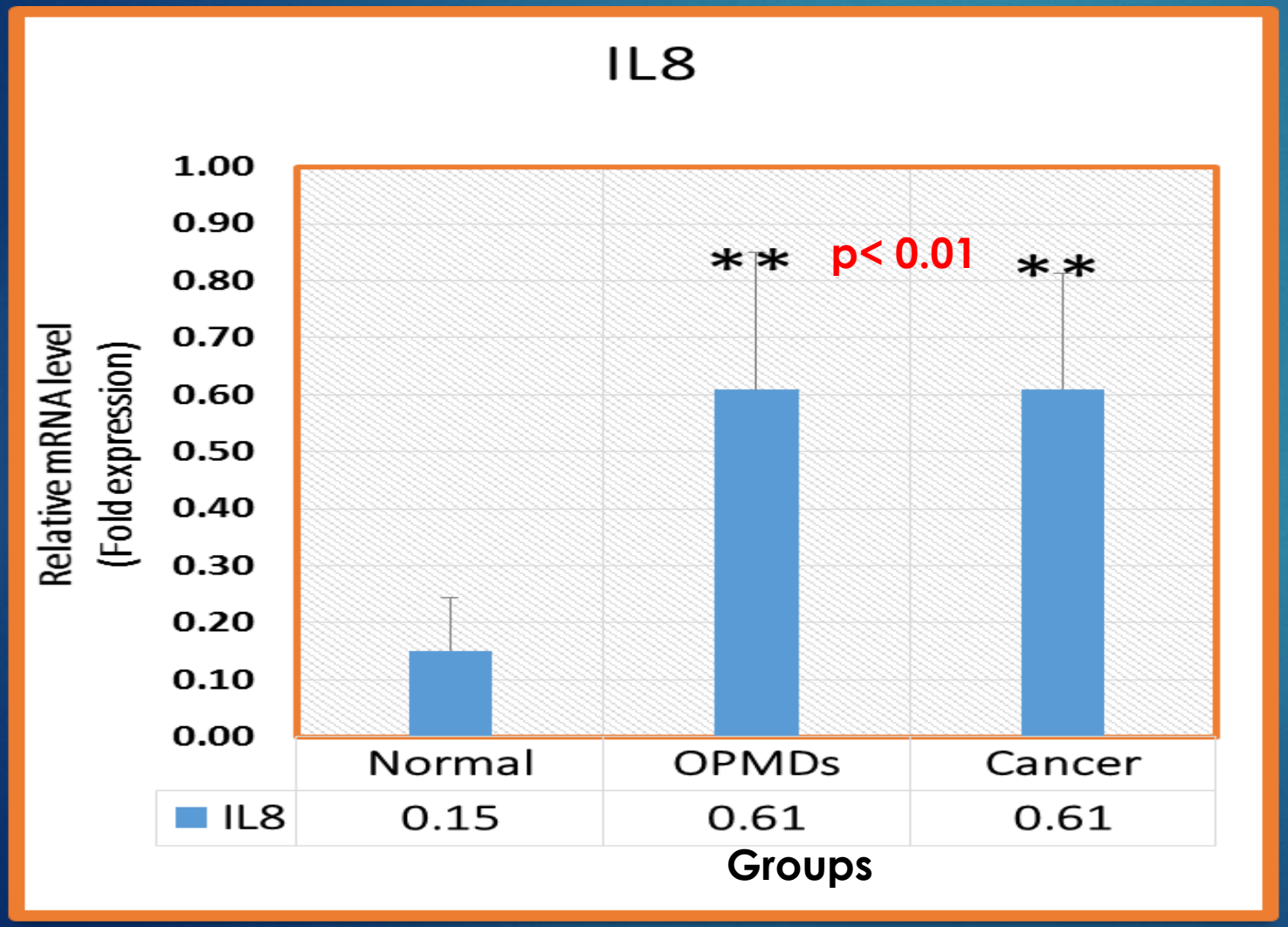
- Up-regulates the inflammatory response.
- signal transduction and proliferation, and apoptosis.

Agree with (Li et al., 2004a) and (Elashoff et al., 2012).

Disagree with (Cheng et al., 2014)

The relative mRNA expression level of IL8

significant increase in both diseased groups each =0.46 fold, control =0.15 fold.



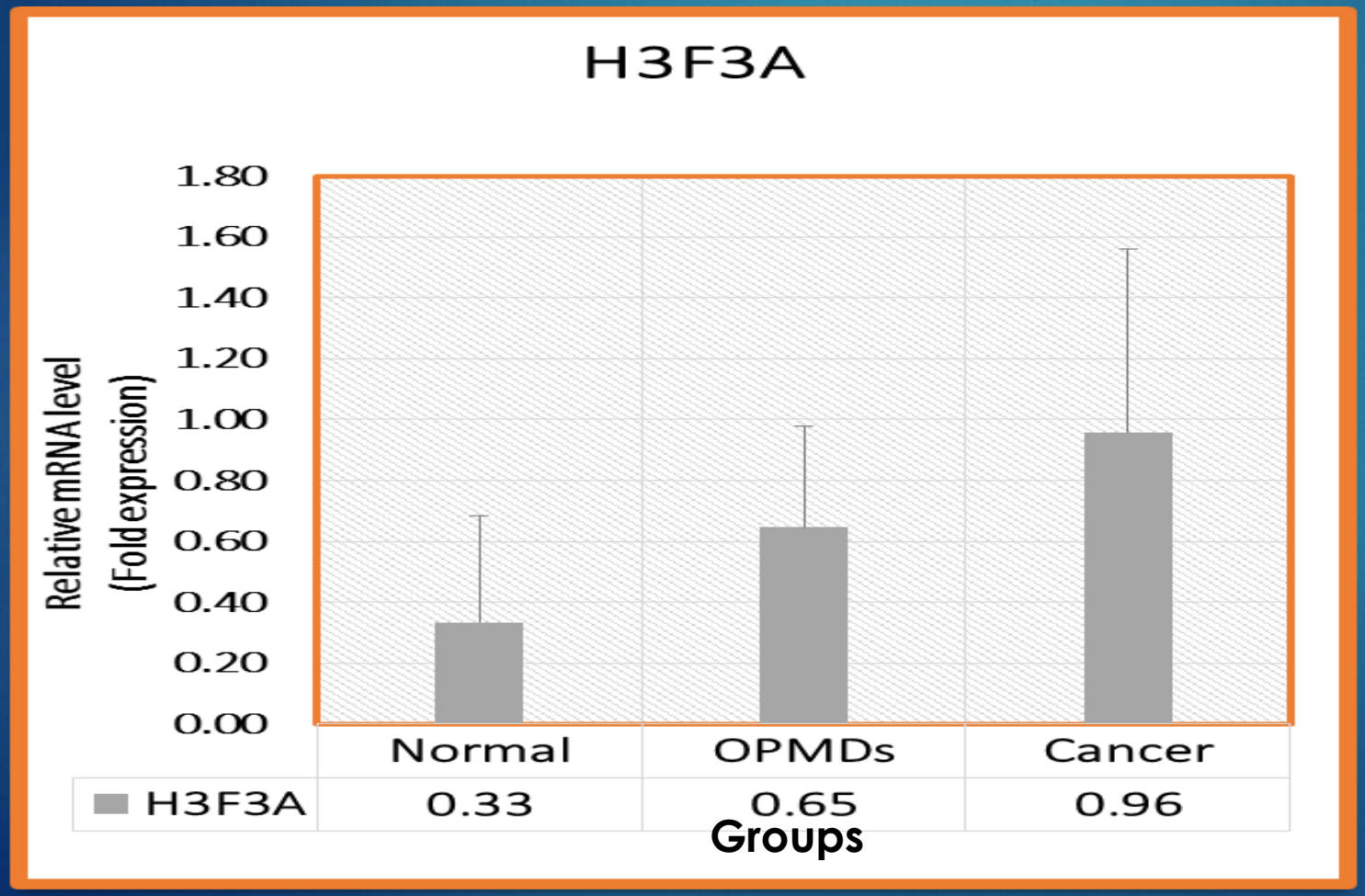
IL8 is a proinflammatory cytokine

- has role in tumor angiogenesis, cell adhesion, immunity, and cell cycle arrest.
- is a significant regulatory factor in tumor microenvironment

Agree with (Li et al., 2004a) , (St John et al., 2004), (Brinkmann et al., 2011) and (Elashoff et al., 2012).

Disagree with (Cheng et al., 2014)

The mean of relative mRNA expression level of H3F3A non significant increase (0.32 and 0.63 fold respectively).



It is a basic nuclear protein and it is important in DNA binding activity.

Agree with (Cheng et al., 2014) in both OSCC and OPMDs study groups

Disagree with (Li et al., 2004a) ,
and (Elashoff et al., 2012)

CONCLUSIONS

The use of salivary transcriptome quantification can be developed as a dependent tool for future disease diagnosis.

1. successfully applied for detecting OMPDs and SCC at early stage.
2. IL1B rather than IL8 to be related to the changes associated with erosive OLP.
3. IL8 seems to be related to malignant transformation and help to identify patients that are at high risk or developing early SCC.
4. H3F3A may be a late events and not useful for detecting early transformation.

THANK YOU