

**Dental Caries Experience and its Association
with *Streptococcus mutans*-*Candida albicans*
Counts in a Group of Egyptian Children**

Thesis

**Submitted to the Department of Pediatric Dentistry and
Dental Public Health - Faculty of Dentistry**

Ain Shams University

In

**Partial fulfillment of the requirements of the Master's degree in
Pediatric Dentistry**

By

Eman Hassan Abdul Megid

B.D.Sc. Ain Shams University

2010

Faculty of Dentistry

Ain Shams University

2018

Supervisors

Dr. Amr Mahmoud Abd el Aziz

Professor and Head of

Pediatric Dentistry and Dental Public Health Department

Faculty of Dentistry

Ain Shams University

Dr. Mariem Osama Mohamed Wassel

Lecturer of Pediatric Dentistry and Dental Public Health

Faculty of Dentistry

Ain Shams University

Dr. Dina Mohammad Erfan

Assistant Professor of Medical Microbiology and Immunology

Faculty of Medicine

Ain Shams University

Acknowledgment

First of all, all praise is due to **Allah**, the most beneficent and the most merciful for the blessings, guidance and generosity he bestowed on me throughout my life.

I would like to express my deepest gratitude and admiration to **Dr. Amr Mahmoud Abd El-Aziz**, Professor and Head of the Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Ain Shams University for his continuous guidance throughout the course of this study.

My most profound and heartfelt gratitude goes to **Dr. Mariem Osama Mohamed**, Lecturer, Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Ain Shams University, for her guidance, precious time and constant support. I am particularly thankful for her expert opinion and valuable feedback. It was an honor working under her supervision.

I would like to sincerely thank **Dr. Dina Mohammad Erfan**, Associate Professor, Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University, for steering me in the right direction.

I am forever grateful to my professors, colleagues and staff members of the Pediatric Dentistry and Dental Public Health Department for their tremendous help and genuine support. Last but not least, I would like to extend my deepest thanks to all the participants in this study. Without them, this work would not have been possible.

This work is dedicated to...

My lovely family, for their endless love and support.

My sister, who has always been there for me.

*My wonderful friends: Amany mostafa, Dina Darwish, Dina
ELDisouki, Reham Ali and Samah el Nahass.*

List of Contents

<u>Title</u>	<u>Page No.</u>
List of Tables.....	I
List of Figures.....	IV
List of Abbreviations.....	VIII
Introduction.....	1
Review of Literature.....	3
Aim of the Study.....	31
Subjects and Methods.....	32
Results.....	72
Discussion.....	96
Summary.....	110
Conclusions.....	114
Recommendations.....	115
References.....	116
Appendices.....	-
Arabic Summary.....	-

List of Tables

<u>Table No.</u>	<u>Table Title</u>	<u>Page No.</u>
1	Composition of mitis salivarius agar media	44
2	Composition of sabouraud dextrose agar media	45
3	QIAmp® DNA Mini kit reagents	51
4	<i>C.albicans</i> -specific primers	56
5	PCR reaction mixture components	56
6	Thermal cycler conditions for amplification	56
7	Reverse-Transcription Reaction Components	66
8	Primer sets of <i>C.albicans</i> Sap1, Sap2, and ACTIN genes	67
9	Age and gender distribution in group A (ECC group) and group C (age-matched control)	72
10	Age and gender distribution in group B (dental caries group) and group D (age-matched control)	73
11	Parental education and employment in group A (ECC group) and group C (age-matched control)	74

12	Parental education and employment in group B (dental caries group) and group D (age-matched control)	75
13A	Nursing habits of group A (ECC group) and group C (age-matched control)	76
13B	Dietary habits of group A (ECC group) and group C (age-matched control)	77
14	Dietary habits of group B (dental caries group) and group D (age-matched control)	78
15	Tooth brushing frequency of group A (ECC group) and group C (age-control)	79
16	Tooth brushing frequency of group B (dental caries group) and group D (age-matched control)	80
17	Caries scores for group A (ECC group) and group B (dental caries group)	81
18	The detection of <i>S.mutans</i> and <i>C.albicans</i> in saliva samples of group A (ECC group) and group C (age-matched control)	82
19	The detection of <i>S.mutans</i> and <i>C.albicans</i> in saliva samples of group B (dental caries group) and group D (age-matched control group)	82
20	Salivary and dentinal detection of <i>S.mutans</i>	84

	and <i>C.albicans</i> in group A (ECC group) and group B (dental caries group)	
21	Salivary <i>S.mutans</i> and <i>C.albicans</i> counts in group A (ECC group) and group C (age-matched control)	85
22	Salivary <i>S.mutans</i> and <i>C.albicans</i> counts in group B (dental caries group) and group D (age-matched control)	86
23	Salivary and dentinal counts of <i>S.mutans</i> and <i>C.albicans</i> in group A (ECC group) and group B (dental caries group)	87
24	The correlation between microbial count and caries score among ECC group (group A)	88
25	The correlation between microbial count and caries score among dental caries group (group B)	91
26	Salivary <i>C.albicans</i> Sap1 and Sap2 expression levels for group A (ECC group) and group C (age-matched control)	93
27	Salivary <i>C.albicans</i> Sap1 and Sap2 expression levels for group B (dental caries group) and group D (age-matched control)	94
28	Salivary and dentinal <i>C.albicans</i> Sap 1 and Sap 2 expression levels for group A (ECC group) and group B (dental caries group)	95

List of Figures

<u>Figure no.</u>	<u>Title</u>	<u>Page no.</u>
1	A 5-year-old child with early childhood caries (group A)	34
2	An 8-year-old child with dental caries (group B)	35
3	A 4-year-old caries free child (group C)	35
4	An 8-year-old caries free child (group D)	36
5	Saliva sample collection	41
6	Cariious dentin sample collection	43
7	Mitis salivarius agar media and bacitracin	45
8	Sabouraud dextrose agar media and cycloheximide	46
9	Samples' vortexing	47
10	Inoculated modified mitis salivarius agar plates in anaerobic jar	48
11	<i>S.mutans</i> colonies on modified mitis salivarius agar (red arrows)	49
12	Candidal colonies on sabouraud dextrose agar (red arrows)	50
13	Candidal colonies under light microscope	50

14	QIAmp® DNA Mini kit reagents	52
15	DNA extraction procedure	54
16	PCR reaction mixture-containing tubes in the thermal cycler (red arrows)	57
17	Materials used for gel electrophoresis	58
18	Samples' loading into the agarose gel	60
19	Gel chamber connected to the power supply	61
20	Ethidium bromide-stained and UV-transilluminated PCR product	61
21	Qiagen miRNeasy® Minikit	63
22	Three phases after QIAzol lysis	64
23	Omni script reverse transcriptase kit	65
24	Omni script reverse transcriptase procedure	66
25	Real-time master mix components	67
26	Real-time PCR system thermal cycling block	68
27	Real-time PCR amplification plot	69
28	Bar chart showing gender distribution in different groups	73
29	Bar chart showing on demand feeding in	77

	group A and group C	
30	Bar chart showing snacking frequency in different groups	78
31	Bar chart showing tooth brushing frequency in different groups	80
32	Bar chart showing the detection frequency of salivary <i>S.mutans</i> and <i>C.albicans</i> in different groups	83
33	Bar chart showing the detection frequency of salivary and dentinal <i>S.mutans</i> and <i>C.albicans</i> in group A and group B	84
34	Bar chart showing salivary <i>S.mutans</i> and <i>C.albicans</i> counts in group A and group C	85
35	Bar chart showing salivary <i>S.mutans</i> and <i>C.albicans</i> counts in group B and group D	86
36	Bar chart showing salivary and dentinal <i>S.mutans</i> and <i>C.albicans</i> counts in groups A and B	87
37	Scatter plot showing the correlation between salivary <i>S.mutans</i> counts and dmft scores	89
38	Scatter plot showing the correlation between dentinal <i>S.mutans</i> counts and dmft scores	89
39	Scatter plot showing the correlation between salivary <i>C.albicans</i> counts and dmft scores	90

40	Scatter plot showing the correlation between salivary <i>S.mutans</i> counts and deft scores	91
41	Scatter plot showing the correlation between dentinal <i>S.mutans</i> counts and deft scores	92
42	Bar chart showing the expression levels of salivary <i>C.albicans</i> Sap1 and Sap2 in group A and group C	93
43	Bar chart showing the expression levels of salivary <i>C.albicans</i> Sap1 and Sap2 in group B and group D	94
44	Bar chart showing the expression levels of <i>C.albicans</i> Sap2 in group A and group B	95

List of abbreviations

<u>Abbreviation</u>	<u>Full term</u>
AAP	American Academy of Pediatrics
AAPD	American Academy of Pediatric Dentistry
AEP	Acquired enamel pellicle
Bp	Base pair
<i>C.albicans</i>	Candida albicans
CFUs	Colony forming units
CI	Confidence interval
Deft	Decayed, missing or indicated for extraction deciduous teeth
Dmft	Decayed, missing or filled deciduous teeth
DMFT	Decayed, missing and filled permanent teeth
DNA	Deoxyribonucleic acid
ECC	Early childhood caries
ECP	Extracellular polysaccharides
EDTA	Ethylene diamine tetra acetic acid
Gtf	Glucosyltransferase
MSA	Mitis salivarius agar

<i>n.s</i>	Non significant
OR	Odds ratio
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT	Reverse transcription
Saps	Secreted aspartyl proteinases
SDA	Sabouraud dextrose agar
S-ECC	Severe early childhood caries
SES	Socio-economic status
sIgA	Secretory immunoglobulin A
<i>S.mutans</i>	Streptococcus mutans
spp	Species
SPSS	Statistical Package for Social Science
UV	Ultraviolet
WHO	World Health Organization

Introduction

Oral health is an integral part of general health and is extremely pivotal in the normal development of a child. Despite the great achievements in oral health globally, dental caries remains a major public health concern among children. It has been regarded as the second most prevalent chronic disease and the most common oral disease worldwide ^(1, 2).

Early childhood caries (ECC) is a virulent form of dental caries. It is a disconcerting type of tooth decay that most frequently affects underprivileged preschool children. The early onset and rapid progression of ECC result in rampant destruction of the primary teeth ⁽³⁾.

Dental caries is a multi-factorial disease. Identifying the specific risk factors of the disease is fundamental for the effective early identification of 'at risk' children; it is the first milestone in reducing the incidence and severity of dental caries in future generations. Among the commonly recognized risk factors, the microbial factor is the most challenging ⁽⁴⁾.

The oral cavity represents a highly diverse, highly dynamic microbial community with over 700 microbial species (spp.). Mutans Streptococci; including *Streptococcus mutans* (*S.mutans*) and *Streptococcus sobrinus* have been always considered significant contributors to tooth decay. Other contributors are non-mutans Streptococci, *Lactobacillus*, *Actinomyces*, *Bifidobacterium*, and *Veillonella* spp ^(5,6).