

C-REACTIVE PROTEIN, PERIODONTITIS AND SYSTEMIC INFLAMMATION

A report submitted to the University of Adelaide in partial fulfillment of the requirements of the Degree of Doctor of Clinical Dentistry (Periodontology)

Emma Dominique Megson BSc (UNSW) BDent (Hons) (USyd)



Table of Contents

Abstract.....	iii
Declaration.....	iv
Acknowledgements.....	v
Chapter One. Literature Review of C-reactive Protein.....	1
1.1 Introduction.....	1
1.2 What is C-reactive Protein?	1
1.3 Source	2
1.4 Structure.....	3
1.5 Forms of CRP	4
1.6 Ligand Binding	5
1.7 Receptors	5
1.8 Functional properties	6
1.9 Acute Phase Response	8
1.10 Normal levels of CRP	10
1.11 Measurement of CRP.....	11
1.12 Genetics	11
1.13 CRP is Associated with Systemic Disease	13
1.13.1 Intervention Studies	15
1.13.2 Biological Considerations.....	16
1.14 Systemic Disease is Associated with Periodontal Disease	18
1.14.1 Intervention Studies	19
1.14.2 Biological Plausibility.....	21
1.15 Periodontal Disease is Associated with CRP.....	23
1.15.1 Cross Sectional Studies of CRP in Saliva and GCF	24
1.15.2 Cross Sectional Studies for Serum CRP and Periodontitis.....	26
1.15.2.1 No Correlation with CRP.....	26
1.15.2.2 Insufficient Adjustment for Confounders	26
1.15.2.3 Systemic Medical Conditions	26
1.15.2.4 Non-traditional Measures of Periodontal Disease	27
1.15.2.5 Epidemiological Studies	27
1.15.2.6 Systemically Healthy Subjects.....	28
1.15.2.7 Edentulousness.....	29
1.15.3 Serum CRP and Periodontal Therapy Longitudinal Studies	29
1.15.3.1 Time Course of Changes in Serum CRP Following Periodontal Therapy	29

1.15.3.2 Non-surgical Therapy	30
1.15.3.3 Surgical and Non-surgical Therapy	31
1.15.3.4 Adjunctive Local Antibiotics	31
1.15.3.5 Adjunctive Systemic Antibiotics	32
1.15.3.6 Host Modulation	32
1.15.3.7 Dental Clearance	32
1.15.3.8 Summary	33
1.15.4 Longitudinal Studies of Serum CRP and Periodontal Disease Activity	35
1.15.5 Biological Plausibility	36
1.16 Conclusion	38
1.17 References	40
Chapter Two. C-reactive Protein in Gingival Crevicular Fluid may be Indicative of Systemic Inflammation	73
2.1 Introduction	74
2.2 Methods	75
2.2.1 Study Population	75
2.2.1.1 Patients	75
2.2.2 Sample Collection, Processing and Analysis	75
2.2.2.1 Gingival Crevicular Fluid (GCF) Collection and Analysis	75
2.2.2.2 Immunohistochemistry for C-reactive protein	76
2.2.2.3 Real-Time Polymerase Chain Reaction for CRP gene detection	77
2.2.3 Statistical Analysis	78
2.3 Results	79
2.3.1 Subject demographics and sample collection	79
2.3.2 Detection of CRP in GCF samples	79
2.3.3 Immunohistochemical staining for CRP in gingival tissue	81
2.3.4 Real-time CRP analysis of gingival tissue for CRP mRNA gene expression	81
2.4 Discussion	81
2.5 References	85
Tables and Figures	91
Chapter Three. Addendum	94
3.1 Addendum	94
3.2 References	96

Abstract

Background and Aim: Periodontitis is associated with elevated C-reactive protein (CRP) in both serum and gingival crevicular fluid (GCF). CRP is an acute phase protein, the levels of which closely follow inflammatory disease activity. CRP is used as a risk predictor for cardiovascular events, including myocardial infarction. Periodontitis is associated with an increased risk cardiovascular disease. The nature of the relationship between periodontitis and cardiovascular disease is unclear, but may involve systemic inflammation as measured by CRP. Although the liver is the primary source of CRP, extra-hepatic production of CRP has been reported. Local production of CRP in the periodontal tissues may contribute to serum levels. This study aimed to determine whether CRP in GCF is produced locally in the gingivae.

Materials and Methods: Gingivae and GCF were collected from non-periodontitis and periodontitis sites. Presence of CRP in gingivae was assessed by immunohistochemistry. CRP in GCF was measured using ELISA. Gene expression for CRP in gingivae was determined using real-time polymerase chain reaction.

Results: CRP was found in both the gingivae and GCF. No gingivae had detectable amounts of CRP mRNA. Not all patients with periodontitis had detectable levels of CRP in the GCF. Some non-periodontitis patients had detectable levels of CRP in the GCF.

Conclusion: CRP in the GCF appears to be of systemic origin, and therefore may be indicative of systemic inflammation from either a periodontal infection or inflammatory disease elsewhere. CRP in the GCF may be a substitute measure for serum CRP. The correlation between levels of CRP in GCF and serum requires validation in future studies.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Emma Dominique Megson and, to the best of my knowledge and belief, contains no material previously published or written by another person except where due reference has been made in the text.

The input of the co-authors to this work was mainly advisory and I carried out the bulk of the laboratory procedures and all the writing of this manuscript.

I give consent to this copy of the thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Published works:

Megson E, Fitzsimmons T, Dharmapatni K, Bartold PM. C-reactive protein in gingival crevicular fluid is indicative of systemic inflammation. *Journal of Clinical Periodontology* 37:797-804; 2010.

Declared by: Dr Emma Megson

Witnessed by: _____

Date:

Date:

Acknowledgements

This study was supported by a grant from the National Health and Medical Research Council of Australia (Project Grant # 565341).

I wish to thank my supervisor Professor Mark Bartold for his guidance and feedback.

I was very lucky to be expertly supported in my laboratory work by Dr Tracy Fitzsimmons and Dr Kencana Dharmapatni. Their knowledge and enthusiasm is much appreciated.

Thank you to Mr Dale Caville for his help with imaging the histological sections.

Thank you to Ms Catherine Offler for her assistance in the final editing of this manuscript.

I am very grateful for the excellent help from my postgraduate colleagues, Dr Raymond Chan, Dr Chris Bates, Dr Geoff Harvey, Dr Kere Kobayashi and Dr Tina Choo in sample collection, and also for their friendship over the course of the program.

I could not have withstood the rigors of the program without support from my partner Dr Raymond Chan. His dedication to excellence has been an inspiration for me both personally and professionally.