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IMMUNOPHATOGENESIS OF THE ORAL EPITHELIAL MUCOSAL DESTRUCTION DUE TO MONOMER METHYL METHACRYLATE EXPOSURE THROUGH HYPERSENSITIVITY REACTION

(IMUNOPATOGENESIS KERUSAKAN JARINGAN MUKOSA MULUT AKIBAT PAPARAN MONOMER METIL METAKRILAT MELALUI REAKSI HIPERSENSITIVITAS)

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Abstract

A basic material of polymethyl methacrylate (PMMA) acrylic resins is methyl methacrylate (MMA) monomer and widely used in dental medicine. It is primarily used for removable orthodontic, partial and full denture appliances, and also for dental fillings. The facts and results of the previous studies showed that MMA might act as irritant in certain concentration, and also as immunogen or allergen. This study examined the immunopathogenesis of oral mucosal tissue destruction in patients exposed to MMA, by detecting the concentrations of plasma IL-4, IFN-γ, TNF-α, serum IgG and IgE specific to MMA that mediated irritation and allergic reactions. The general objective of this research was to examine the immunopathogenesis of oral mucosal tissue destruction in patients exposed to MMA. The study used observasional case control study design. Laboratory tests for all samples were assessing plasma IL-4, IFN-γ and TNF-α using direct sandwich ELISA technique. Serum IgG and IgE specific to MMA were assessed by indirect ELISA. It was concluded that MMA is immunogenic in patients exposed to MMA that can induce IgG anti-MMA. Furthermore, this study also proved that immunopathogenesis of oral mucosal tissue destruction in patients exposed to MMA did occur through irritation and type I hypersensitivity reaction mediated by IgE, but occurred through type IV hypersensitivity reactions.

Key words: methyl methacrylate, irritation, hypersensitivity, oral epithelial mucosa

INTRODUCTION

Methyl methacrylate (MMA) monomer is a basic material of polymethyl methacrylate (PMMA) acrylic resins widely used in dental medicine. It is primarily used for removable orthodontic appliances, partial and full denture appliances, and also for dental fillings. Many clinical facts showed that MMA may cause irritation and hypersensitivity reaction in oral mucosa with some clinical symptoms such as hyperemia, mucosal edema, painful oral mucosa, and burning mouth.[1-4] The incidence of clinical symptom in patients exposed to MMA is 0.5-1%.[1] However, immunopathogenesis of oral mucosal tissue destruction in patients exposed to MMA has not been clear.

MMA penetration into mucosal tissue is possible, because its chemical structure is lipophylic that enables MMA to penetrate the barrier of oral mucosal tissue. The facts and results of the previous studies showed that MMA might act as irritant in certain concentration, and also as immunogen or allergen.[5-9] However, the immunopathogenesis of oral mucosal tissue destruction through irritation and hypersensitivity reaction in patients exposed to MMA has not been clear.

The preliminary study proved that MMA can induce a secondary immune response in local rabbit immunized with MMA, by examining the IgG anti-MMA production pattern in certain period.[10,11] This study examined the immunopathogenesis of oral mucosal tissue destruction in patients exposed to

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MMA, by detecting the concentrations of plasma IL-4, IFN-γ, TNF-α, serum IgG and IgE specific to MMA that mediated irritation and hypersensitivity reactions.

Hopefully, the results of this study could be as scientific information about irritation and hypersensitivity reaction in patients exposed to MMA. So, this study can be used as a reason to find a new material for sensitive patients to MMA, as alternative dental material.

MATERIALS AND METHODS

This study used observasional case control study design. Samples were devided into two groups, i.e. control and case group.[11]

Control group was MMA unexposed patients without inflammation symptom. Inclusion criteria for a control group, patients who were no MMA exposed or no denture wore are man or woman with no clinical symptoms: hyperemia; local inflammation, pain, and *burning mouth*, ulcer, no anti-allergic drug consuming. Case group was first MMA exposed patients with local inflammation symptoms. Inclusion criteria for a case group, patients who were MMA exposed for the first time are women or man who wore the partial or full denture with the age 35-60 years; normal weight; with clinical symptoms: hyperemia; local inflammation, vesicular, and *burning mouth*, ulcer, no anti-allergic drug consuming. Total samples of each group were 8 patients from Airlangga University Prosthodontic Dental Clinic.

Laboratory tests for all samples were assessing plasma IL-4, IFN-γ and TNF-α using direct sandwich ELISA technique. Serum IgG and IgE specific to MMA were assessed by indirect ELISA (method by BrenderMed System©).

RESULTS

Clinical identification has been found in patients who wore the full denture were hyperemia, mucosal edema, vesicular, and burning mouth (Figure 1).

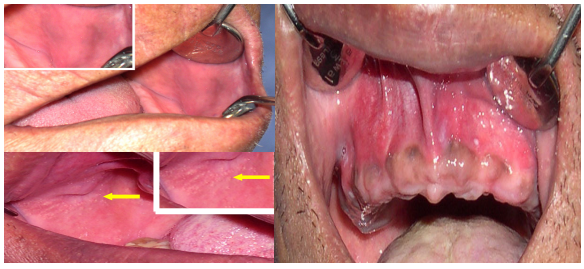

Figure 1. Oedematous, hyperemia, vesicula, and burning mouth

The result of IgG specific to MMA detection showed that the case group has positive result with absorbance scores between 0.035-0.0360 (Table 1).

Table 1. Absorbance specific IgG in patients exposed to MMA

No.	Patient absorbance specific IgG at 450 nm	Mean control	Positif point	Patient Pos /Neg
1.	0,0350	0,01	0,02	Pos
2.	0,0300	0,01	0,02	Pos
3.	0,0360	0,01	0,02	Pos
4.	0,0530	0,01	0,02	Pos
5.	0,0450	0,01	0,02	Pos
6.	0,0510	0,01	0,02	Pos
7.	0,0410	0,01	0,02	Pos
8.	0,0510	0,01	0,02	Pos

Table 2. Absorbance specific IgE in patients exposed to MMA

No.	Patient absorbance specific IgE at 450 nm	Mean control	Positif point	Patient Pos /Neg
1.	0,1110	0,13	0,26	Neg
2.	0,1270	0,13	0,26	Neg
3.	0,1280	0,13	0,26	Neg
4.	0,1450	0,13	0,26	Neg
5.	0,1280	0,13	0,26	Neg
6.	0,1540	0,13	0,26	Neg
7.	0,1590	0,13	0,26	Neg
8.	0,0991	0,13	0,26	Neg

Table 3. Consentration plasma IL-4 (pg/ml) between patienst exposed to MMA and controls

No.	Plasma	
	Controls	Patients
1.	10,43	58,70
2.	14,78	66,65
3.	24,35	83,48
4.	23,91	82,17
5.	24,78	70,00
6.	20,87	60,00
7.	20,00	58,26
8.	23,48	59,13

Furthermore, the IgE spesific to MMA has negative result in case group. Absorbance scores IgE specific to MMA in case group are between 0,0991-0,1590.

Edisi Cetak Dentika Dental Journal, Juli 2009 (ISSN: 1693-671X)

Table 4. Independent t test for plasma IL-4 between patients exposed to MMA and controls

	Plasma	
	Controls	Patients
Mean	20,32 pg/ml	67,30 pg/ml
Standard deviation	5,18 pg/ml	10,46 pg/ml
t count	11,379	
p	0,01 *	

*Significant (p< 0,05)

Table 5. Consentration plasma TNF-α (pg/ml) between patients exposed to MMA and controls

No.	Plasma	
	Controls	Patients
1.	11,30	23,67
2.	9,16	23,98
3.	9,93	23,67
4.	11,94	24,43
5.	10,69	28,41
6.	10,38	29,17
7.	9,16	28,41
8.	9,16	28,86

Table 6. Independent t test for variabel plasma TNF-α between patients exposed to MMA and controls

	Plasma	
	Controls	Patients
Mean	35,52 pg/ml	90,77 pg/ml
Standard deviation	2,92 pg/ml	20,45 pg/ml
t count	16,371	
p	0,01*	

*Significant (p< 0,05)

Table 7. Consentration plasma IFN-γ (pg/ml) in patients exposed to MMA and controls

No.	Plasma	
	Controls	Patients
1.	54,76	84,76
2.	52,38	81,83
3.	55,24	95,71
4.	53,33	94,29
5.	52,86	82,86
6.	57,17	80,48
7.	53,81	78,10
8.	50,00	79,05

The plasma IL-4 concentration in case group between 189.13-253.04 pg/ml (Tabel 3) and this is significantly upregulated (p<0.05) (Table 4). Although, plasma IL-4 significantly upregulated, but there is no positive result of IgE specific to MMA.

The plasma TNF-α concentration in case group is higher than in control group. The plasma TNF-α concentration in case group is between 23.67- 29.17 pg/ml and in control group is between 9.16 -11.94 pg/ml. This is significantly different between two groups (p<0,05) (Table 5 and 6).

Furthermore, the plasma IFN-γ concentration in case group is between 78.10 – 95.71 pg/ml and in control group is between 50.00 – 57.17 pg/ml. T-test analysis showed this is significantly upregulated (p<0,05) (Table 7 and 8).

Table 8. Independent t test for plasma IFN-γ between patients exposed to MMA and controls

	Plasma	
	Controls	Patients
Mean	35,52 pg/ml	90,77 pg/ml
Standard deviation	2,93 pg/ml	20,46 pg/ml
t count	12,378	
p	0,01 *	

*Significant (p< 0,05)

DISCUSSION

Irritant reaction or contact hypersensitivity, previously thought to be monomorphous process, is now considered a complex biological syndrome with a diverse pathophysiology, natural history and clinical appearence. Numerous factors determine whether a particular substance will caused irritant and inflamation in given individual. Likewise, the type of exogenous stimulus may influence the reaction. Although certain topically applied irritancy by stripping of the skin exhibited no inflammatory cell infiltration during initial 24 hours.[13,14]

The role of cytokines has been known as phathomechanism of cell-mediated hypersensitivity contact dermatitis and contact irritant. The cytokines regulate each other by competition, interaction and mutual induction in series of lymphokine cascades and circuits with possitive or negative feedback effect.[13,14]

The result of this study showed that MMA can induce immune response with the production of IgG specific to MMA. It means that MMA is immunogenic in patients exposed to MMA (Table 1).

Previous study reported that membrane disturbance caused by monomer MMA. MMA showed

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liposomes changes in liposomes characterized by membrane disturbance. It was suggested that MMA intercalated into the cellular membrane and moved through their lipid phase and injured the cells in low concentation. This finding showed a cooperativity between methyl chain and liposomes of membrane lipid bilayer. However, MMA penetrates deeply into the interior membrane due to lipophylicity and caused injury to cells.[16,17]

Preleminary in vivo study proved that MMA can induce immune response by characterizing IgG specific to MMA in local rabbit after immunisizing with MMA. First booster (day 28 after first immunization) increased IgG anti-MMA production and achieved its peak on day 42, after that began to decrease gradually until day 49. Second booster given on day 52 increased IgG anti-MMA production and achieved its peak on day 80, and after that began to decrease gradually until day 87. The peak of IgG anti-MMA production after the second booster was higher than the first booster.[10,11]

Methyl methacrylate could be conjugated to protein host as protein carrier. Conjugation of MMA with protein carrier through non-covalen amino hydophobic acid, i.e. alanin, leusyn, tyrosin, fenylalanin, and valyn.[18,19]

This study showed that IgE anti-MMA was negative in patients exposed to MMA (Table 2). Plasma IL-4 in patients exposed to MMA were significantly upregulated ($p<0.05$) (Table 3 and 4). Although, plasma IL-4 significantly upregulated, but there was no positive result of IgE specific to MMA. It can be concluded that immunopathogenesis of oral mucosal tissue destruction in patients exposed to MMA did not occur through type I hypersensitivity reaction mediated by IgE. The profile of secreted cytokines is highly dependent on the particular type of T cells in hypersensitivity reaction, it seems that this specific response of T cells to antigenic challenge defines the nature of the immune reponse.[20,21] In 1986 Mosmann et al. (cit. Effendy *et.al.*)[13] began a conconseptual revolution in immunology by divining T helper (Th) cells into two populations with contrasting and cross-regulating cytokine profile: Th1 and Th2 cytokine. The new paradigm was accepted in every area of immunologic and infectious disease. For instance, contact sensitivity has generally been regarded as specific Th1-mediated process.[22-25]

To day, however, there is good evidence that both Th1 and Th2 cytokines, for example, are primarily involved in sensitivity contact, suggesting that certain prior distinctions in molecular mechanisms of cell-mediated delayed type hypersensitivity or sensitivity contact requires revisiting.[15,23]

The results of this study were significantly up-regulated TNF-α ($p<0,05$) (Table 5 and 6). TNF-α secretion could be upregulated by type IV and irritant reaction. It means that MMA caused delayed type hypersensitivity (type IV hipersensitivity) and suggested that upregualted via protein kinase C-dependent increase in promoter activity or induced keratinocytes without intermediate Langerhans cell (LC)-derived signals.[26]

Furthermore, the results of the study showed that there were also significantly upregulated IL-4 and IFN-γ (Table 3 and 7). Enk and Katz[27] showed a distinct cascade of epidermal cytokines in iritant reaction caused by irritant (i.e. chemical substance) when compared with that in early phase of hypersensitivity reaction (type I hypersensitivity) induced by allergen. Kondo *et. al.*[28] reported that the upregulated TNF-α, because of LC-derived cytokine has been thought to be specific for sensitivity contact. Furthermore, it explaned that allergen activated lymph node cells (LNC).

If immunopathogenesis of oral mucosal tissue destruction in patients exposed to MMA happened through irritation reaction, there would be significantly upregulated ($p<0.05$) of plasma TNF-α, without significantly upregulated ($p>0.05$) of IL-4 and IFN-γ. However, the results of this study showed that there were significant upregulated of plasma TNF-α, IL-4 and IFN-γ.

Keratinocytes are not only involved in irritant reaction but also in hypersensitivity contact, through the synthesis and the releasing of inflammatory cytokine, chemokines and growth factors. Although there is a distinct pathway between hypersensitivity and irritant reactions, a connecting network at molecular levels between both types of dermatitis contact seems to exist. This may be the reason why numerous similar epidermal cytokines have been involeved in both hypersensitivity and irritant responses. The current state of the epidermal cytokines detected in irritant reactions or in compared to those in hyprsensitivity contact or delayed type hypersensitivity in an in vivo model (cell-cultured keratinocytes) and an in vivo model (epidermis murine).[15, 26, 29-32]

T-cell mediated immune reaction occuring after epicutaneous immunization and challenge with low molecular weight chemicals, i.e, hapten, which covalently bind to discreate amino acid residues on self or exogenous proteins. Hapten-modified protein could then be processed by APC into antigenic peptides, which are transported on the cell suface in association with class I or class II MHC molecules. Epidermal denritic cells, i.e, Langerhans cells (LCs)

Edisi Cetak Dentika Dental Journal, Juli 2009 (ISSN: 1693-671X)

play crucial role in the induction hypersensitivity contact. They capture the hapten (or hatenated protein) in the skin or mucosa and migrate to draining lymph nodes cells recognize a conformational complex formed by hapten-modified peptide within the groove of both MHC class I and class II DC molecules.[14,19,34]

The role of IL-4 has been reported by Bacharier *et. al.*[34], that mice with targeted disruptions of the IL-4 gene. He concluded that IL-10 , but not IL-4 is natural supressant of irritan response as well as hypersensitivity contact. Indeed, IL-10 has been accepted widely as an inhibitor of hypersensitivity contact, but not necessarily of irritant reaction. Recent data implied that IL-4 represented an important down-modulator of hypersensitivity contact, contra-dicting the findings by Bacharier *et. al.*[34]

Recently, mRNA for IL-14 and IFN-γ has been detected only in skin mice with hypersensitivity contact. Both IL-4 and IL-12 may be improtant cytokines in sensitivity contact. Presumably, IL-12 may enhance or induce IL-2 and IFN-γ but inhibit IL-4 in pathogenesis of hypersensitivity contact, respectively.[13, 30, 37] As IL-4 has been known to be a product of Th2 cells, its involement in hypersensitivity contact may probably tell us that Th1 cell cytokines are not solely responsible for the development of sensitivity contact.[34,36]

If immunopathogenesis of oral mucosal tissue destruction in patients exposed to MMA happened through irritation, there would be significant upregulated ($p<0.05$) plasma of TNF-α, without significant upregulated ($p>0.05$) of IL-4 and IFN-γ. However, the results of this study showed that there were significant upregulated plasma of TNF-α, IL-4 and IFN-γ. It can be concluded that immunopathogenesis of oral mucosal tissue destruction in patients exposed to MMA does not happen through irritation.

The results of this study showed that there were significant upregulated ($p<0.05$) of plasma TNF-α and IFN-**γ** in patients exposed to MMA. It can be concluded that oral mucosal tissue destruction happened through type IV hypersensitivity reaction.

The conclusions of this study are MMA is immunogenic in patients exposed to MMA that can induce IgG anti-MMA. Immunopathogenesis of oral mucosal tissue destruction in patients exposed to MMA does not occur through irritation and type I hypersensitivity reaction mediated by IgE, but occur through type II and/or type III, type IV hypersensitivity reactions.

The suggestion of this study is further study should be developed as a diagnostic method for oral mucosal irritation and hypersensitivity reactions in patients exposed to MMA by assessing to oral mucosa tissue that directly exposed to MMA and manifestation of hypersensitivity reaction.

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Edisi Cetak Dentika Dental Journal, Juli 2009 (ISSN: 1693-671X)

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