

CHAPTER IV

RESULTS

The mean ages of the subjects with normal permanent teeth, inflamed permanent teeth, normal primary teeth, and inflamed primary teeth were 17.3 ± 1.1 , 35.4 ± 6.3 , 9.4 ± 0.9 , 6.1 ± 0.7 years (mean \pm SD), respectively. All inflamed permanent teeth were diagnosed with irreversible pulpitis. Of 16 inflamed primary teeth, eight were clinically diagnosed with irreversible pulpitis, whereas the other eight were clinically diagnosed with reversible pulpitis. None of the primary teeth used in this study had root resorption more than 1/3 of the root length. The average duration of dental pain was 56 ± 51 days (mean \pm SD) in the subjects with inflamed permanent teeth and 15 ± 6 days in subjects with inflamed primary teeth. No dental pain was reported in subjects with normal primary or normal permanent teeth.

Aim 1: To investigate whether pulpal inflammation leads to increased levels of Nav1.8 and Nav1.9 expression in the dental pulp of human primary teeth

To quantify the relative amount of nerve fibers in dental pulp, the levels of PGP9.5 compared to β -actin, which is a housekeeper protein, in each sample were measured. The relative values for PGP9.5 in dental pulp of all samples in the same group were pooled for comparison. The pooled data showed that the average relative amounts of nerve fibers in normal permanent teeth ($n = 18$), inflamed permanent teeth ($n = 7$), normal primary teeth ($n = 7$), and inflamed primary teeth ($n = 16$) were 1.017

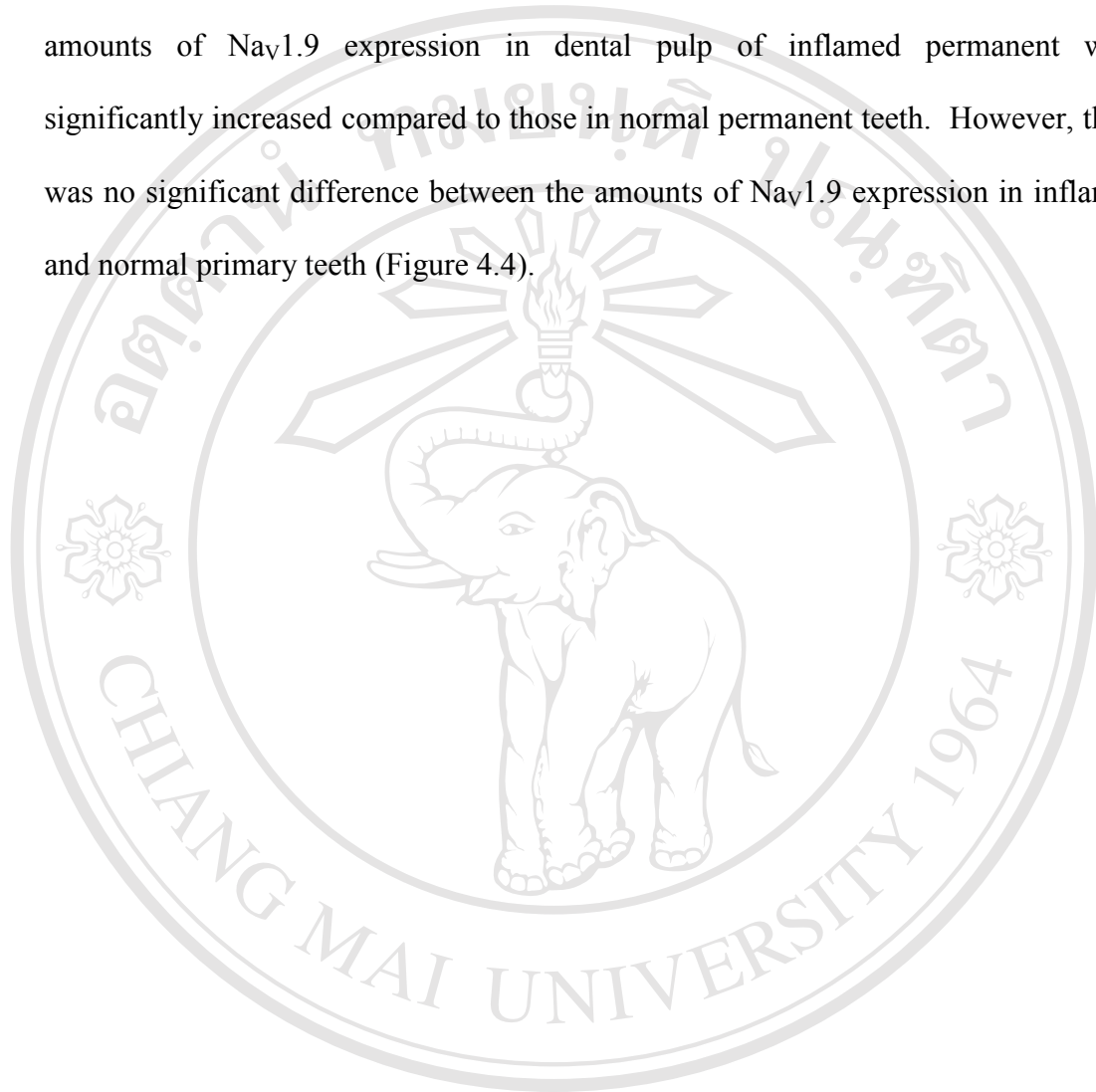
± 0.014 , 1.009 ± 0.040 , 0.939 ± 0.057 , and 1.013 ± 0.047 arbitrary units (mean \pm SE), respectively. There was no significant difference in the relative amounts of PGP9.5 between normal and inflamed dental pulp of both primary and permanent teeth (Figure 4.1).

The levels of pulpal inflammation in this study were indicated by the relative amounts of MMP-9, represented by the ratio of MMP-9 to β -actin. The pooled data for the relative amounts of MMP-9 of samples in the same group showed that the average levels of inflammation in dental pulp of normal permanent teeth ($n = 18$), inflamed permanent teeth ($n = 7$), normal primary teeth ($n = 7$), and inflamed primary teeth ($n = 16$) were 0.832 ± 0.043 , 0.959 ± 0.035 , 0.868 ± 0.059 , and 1.08678 ± 0.05422 arbitrary units (mean \pm SE), respectively. The levels of MMP-9 were significantly higher in inflamed primary teeth ($p < 0.05$) and inflamed permanent teeth ($p < 0.05$) than in normal teeth (Figure 4.2).

The relative amounts of $\text{Na}_v1.8$ expression were taken from the ratio of $\text{Na}_v1.8$ to β -actin and were pooled within the same group. The relative amounts of $\text{Na}_v1.8$ expression in normal permanent teeth ($n = 6$), inflamed permanent teeth ($n = 5$), normal primary teeth ($n = 6$), and inflamed primary teeth ($n = 9$) were 0.696 ± 0.074 , 1.082 ± 0.093 , 0.755 ± 0.036 , and 0.910 ± 0.052 arbitrary units (mean \pm SE), respectively. The relative amounts of $\text{Na}_v1.8$ expression in dental pulp of both inflamed permanent and primary teeth were significantly greater than those in normal teeth ($p < 0.05$) (Figure 4.3).

The ratio of $\text{Na}_v1.9$ to β -actin of each sample was calculated and was pooled within the same group. The relative amounts of $\text{Na}_v1.9$ expression in normal permanent teeth ($n = 8$), inflamed permanent teeth ($n = 5$), normal primary teeth

(n=5), and inflamed primary teeth (n = 9) were 0.850 ± 0.029 , 0.934 ± 0.011 , 0.872 ± 0.035 , and 0.858 ± 0.036 arbitrary units (mean \pm SE), respectively. The relative amounts of Nav1.9 expression in dental pulp of inflamed permanent were significantly increased compared to those in normal permanent teeth. However, there was no significant difference between the amounts of Nav1.9 expression in inflamed and normal primary teeth (Figure 4.4).



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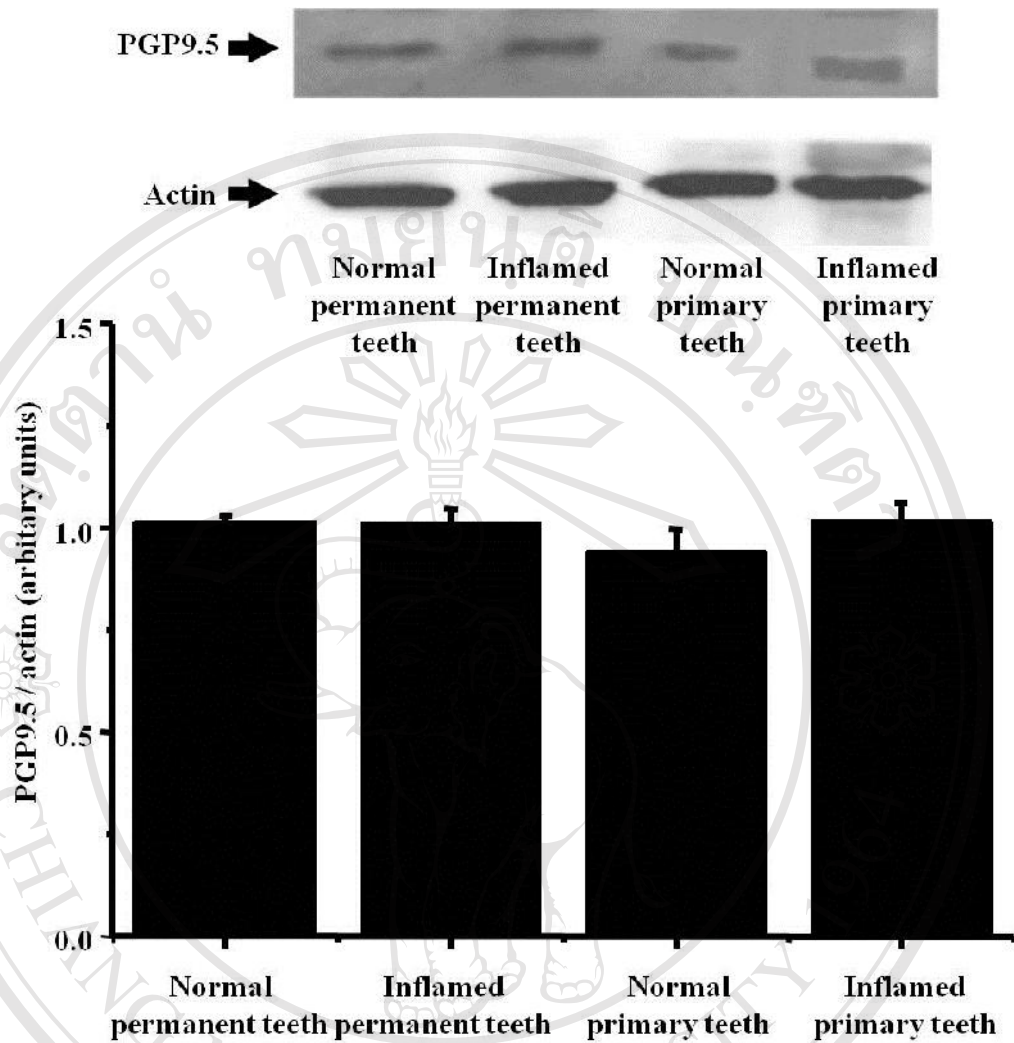


Figure 4.1 Relative amounts of PGP9.5 expression. The picture above the graph shows the expected bands of PGP9.5 (26 kDa) and β -actin (43 kDa) detected by the western blot method, as indicated by the arrows. The band intensity of each sample was normalized with β -actin before being pooled within the same group and plotted as means \pm SE. The graph shows no significant difference in the relative amounts of PGP9.5, normalized with β -actin, in dental pulp of inflamed permanent and primary teeth from that in normal teeth.

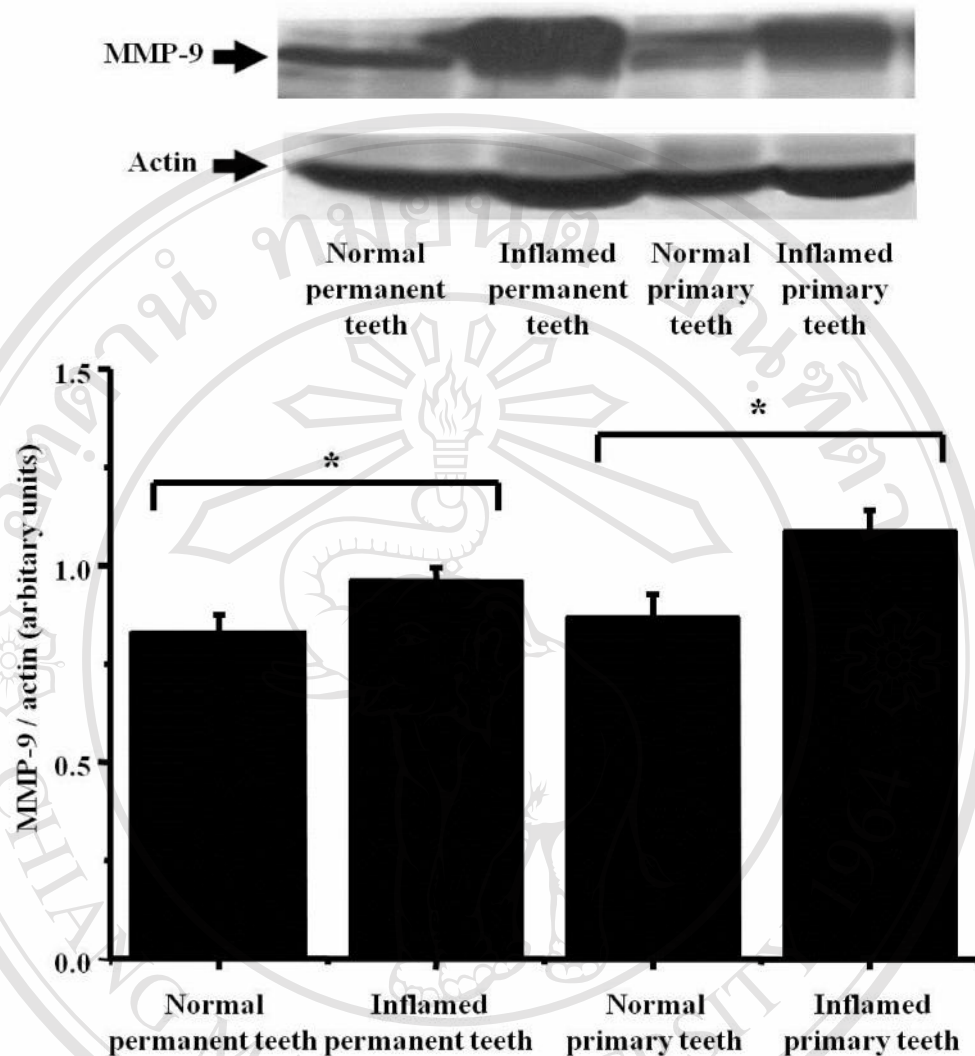


Figure 4.2 Relative amounts of MMP-9 expression. The picture above the graph shows the expected bands of MMP-9 (88 kDa) and β -actin detected by the western blot method, as indicated by the arrows. The band intensity of each sample was normalized with β -actin before being pooled within the same group and plotted as means \pm SE. The graph shows a significant increase in the relative amounts of MMP-9 in inflamed dental pulp of both permanent and primary teeth compared to normal dental pulp (* $p < 0.05$).

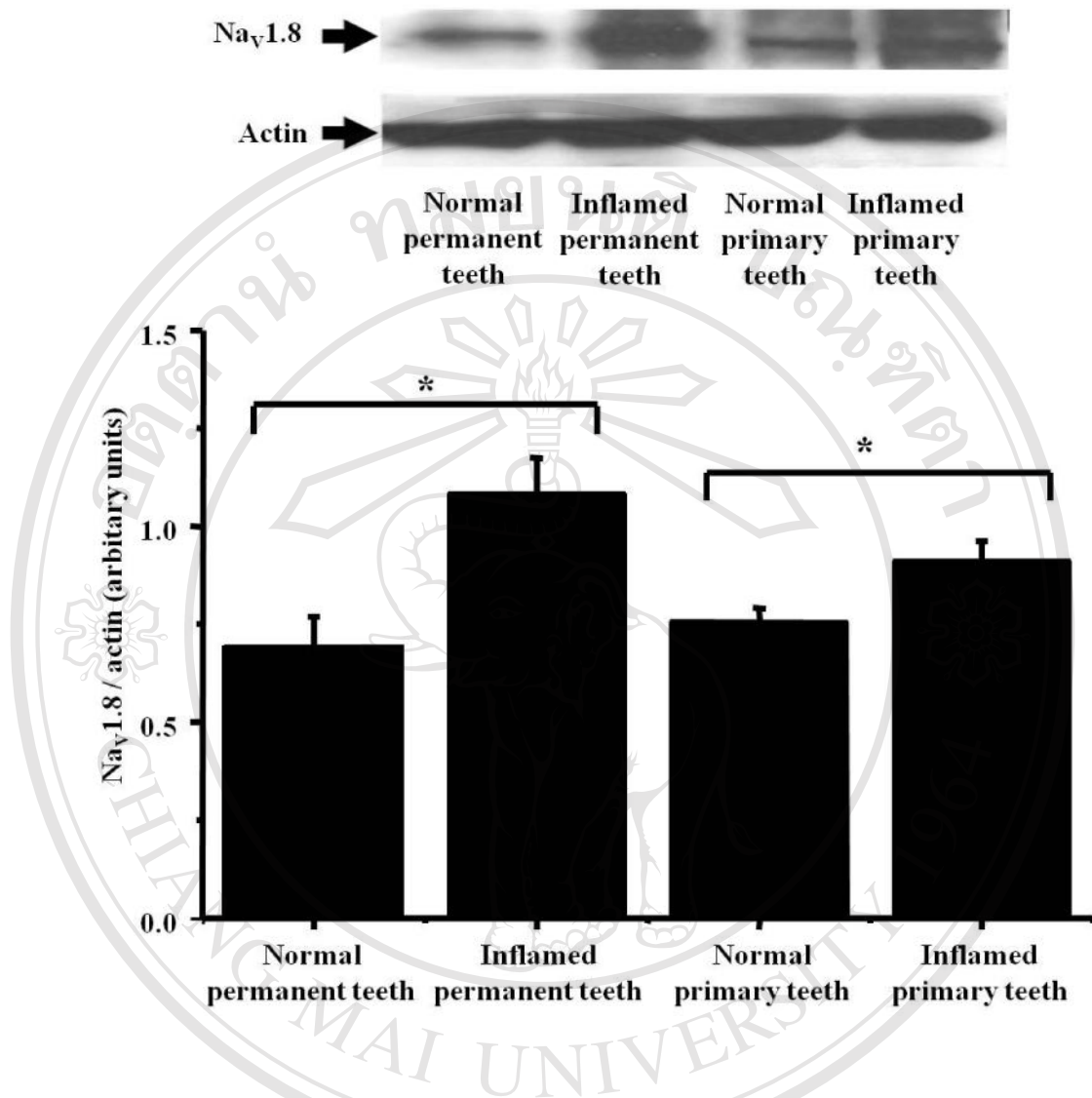


Figure 4.3 Relative amounts of Nav_v1.8 expression. The picture above the graph shows the expected bands of Nav_v1.8 (217 kDa) and β-actin detected by the western blot method, as indicated by the arrows. The band intensity of each sample was normalized with β-actin before being pooled within the same group and plotted as means ±SE. The graph shows that the relative amounts of Nav_v1.8 were significantly increased in the inflamed dental pulp of both permanent and primary teeth (*p < 0.05).

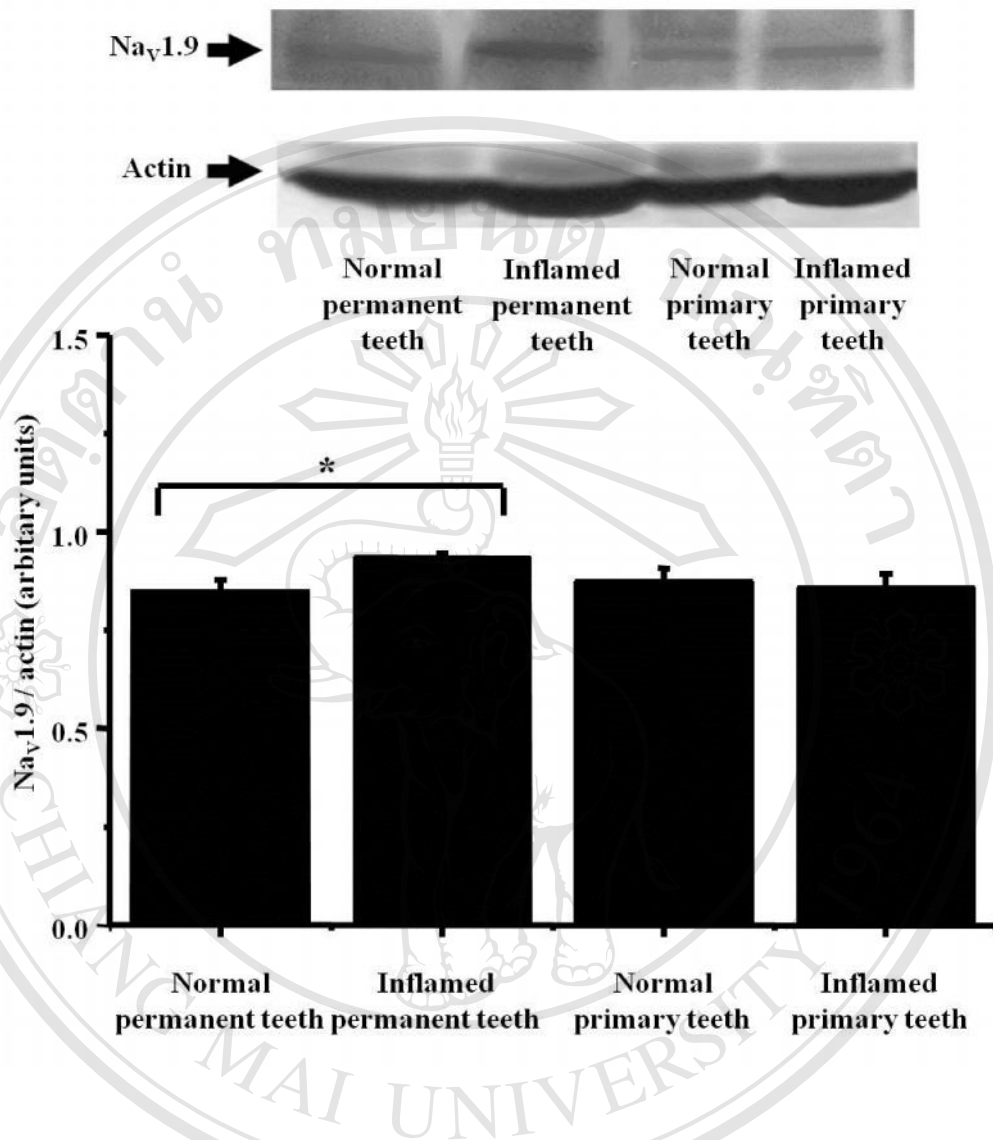


Figure 4.4 Relative amounts of $\text{Na}_V1.9$ expression. The picture above the graph shows the expected bands of $\text{Na}_V1.9$ and β -actin, detected by the western blot method, as indicated by the arrows. The band intensity of each sample was normalized with β -actin before being pooled within the same group and plotted as means \pm SE. The graph shows a significant increase of $\text{Na}_V1.9$ expression in the inflamed dental pulp of permanent teeth (* $p < 0.05$) but there is no significant difference between the relative amounts of $\text{Na}_V1.9$ in normal and inflamed dental pulp of primary teeth.

Aim 2: To investigate whether the levels of Nav1.8 and Nav1.9 expression correlate with the severity of pulpal pain in dental pulp of human primary teeth

In group with permanent teeth, the minimum VAS score was 0, the maximum VAS score was 10, and the average VAS score was 2.2 ± 3.7 (mean \pm SD). The WBFPS score in the group with permanent teeth was 0 for the minimum, 10 for the maximum and 2.1 ± 3.5 for the average. In the group with primary teeth, the minimum VAS score was 0, whereas the maximum VAS score was 10, and the average VAS score was 3.1 ± 4.3 . The WBFPS score in the group with primary teeth was 0 for the minimum, 10 for the maximum, and 3.0 ± 4.3 for the average. The VAS score correlated well with the WBFPS score in both the group with permanent teeth and that with primary teeth ($p < 0.05$, $R = 0.988$ and $p < 0.05$, $R = 1.000$, respectively) as shown in Figure 4.5 and 4.6. For all teeth, the VAS score also significantly correlated with the WBFPS score ($p < 0.05$, $R = 0.993$), as shown in Figure 4.7

The VAS score was only correlated with the expression of Nav1.8 ($p < 0.05$), whereas there was no correlation between VAS score and other protein expression in dental pulp of permanent teeth and there was no correlation between VAS score and any protein expression in group with primary teeth. The data are shown in Table 4.1

The correlation between WBFPS score and protein expression was the same as the correlation between VAS score and protein expression. There was the correlation between WBFPS score and Nav1.8 expression in the group with permanent teeth ($p < 0.05$), but no correlation was found between WBFPS score and other protein expression in the group with permanent teeth or between WBFPS score and any protein expression in the group with primary teeth. The data are shown in Table 4.2

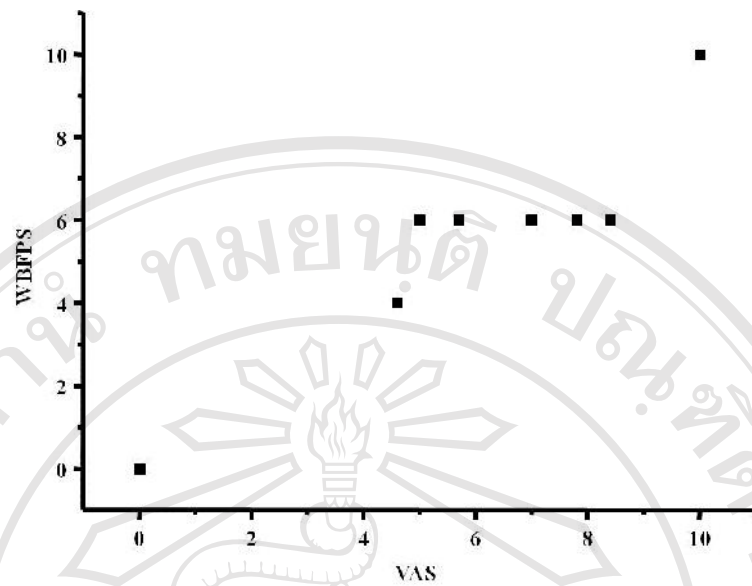


Figure 4.5 The correlation between VAS and WBFPS scores in the group with permanent teeth. The graph shows a significant correlation between the VAS and WBFPS scores ($n = 31$, $p < 0.05$, and $R = 0.988$).

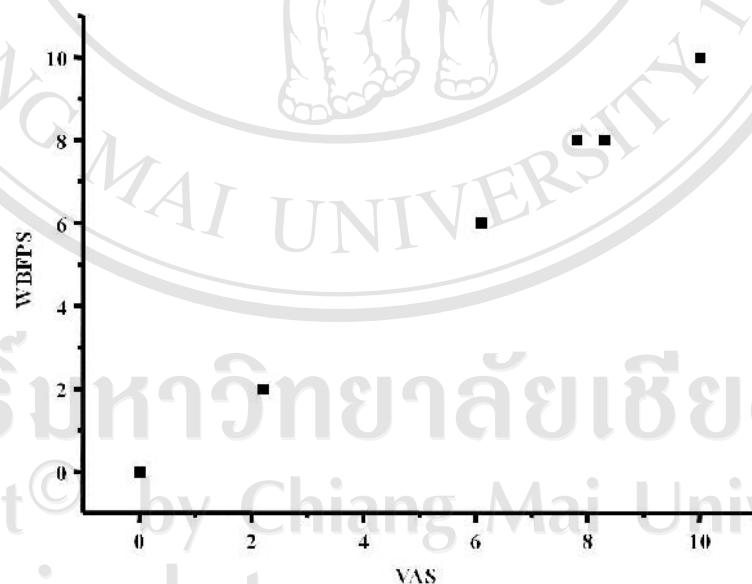


Figure 4.6 The correlation between VAS and WBFPS score in the group with primary teeth group. The graph shows significant correlation between the VAS and WBFPS scores ($n = 21$, $p < 0.05$, and $R = 1.000$).

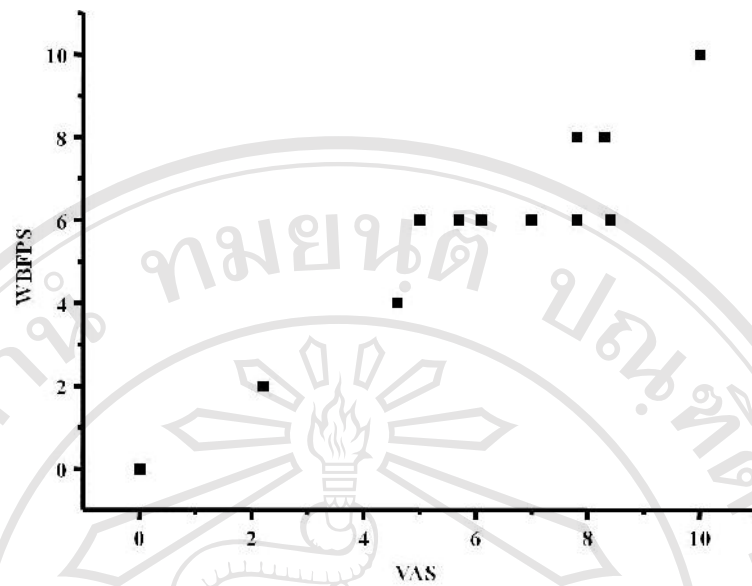


Figure 4.7 The overall correlation between the VAS and WBFPS scores. The graph shows significant correlation between the VAS and WBFPS scores in all subjects ($n = 52$, $p < 0.05$, and $R = 0.998$).

Table 4.1 The correlation between VAS score and protein expression.

Permanent teeth			Primary teeth		
Correlation between VAS and protein expression	Correlation coefficient	p value	Correlation between VAS and protein expression	Correlation coefficient	p value
PGP9.5	-0.131	0.534	PGP9.5	0.286	0.221
MMP-9	0.417	0.060	MMP-9	0.453	0.059
Nav1.8	0.674	0.023*	Nav1.8	0.361	0.204
Nav1.9	0.467	0.108	Nav1.9	-0.340	0.280

* Significant correlation ($p < 0.05$)

Table 4.2 The correlation between WBFPS score and protein expression.

Permanent teeth			Primary teeth		
Correlation between WBFPS and protein expression	Correlation coefficient	p value	Correlation between WBFPS and protein expression	Correlation coefficient	p value
PGP9.5	0.004	0.984	PGP9.5	0.169	0.488
MMP-9	0.314	0.166	MMP-9	0.475	0.054
Nav1.8	0.698	0.017*	Nav1.8	0.361	0.204
Nav1.9	0.482	0.095	Nav1.9	-0.331	0.320

* Significant correlation ($p < 0.05$)