Chapter 4

RESULTS

During 9-month study peroid (from April 1 to December 31, 1995), 32 case samples were collected consecutively, in accompany with 30 control samples. All Green pit viper bites were dignosed by identification of snakes that were brought with the patients. All have at least a sign of envenomation ie. fang mark, blister or necrosis. Control subjects are healthy medical students and residents in Internal Medicine.

Control Group (30 subjects)

Twenty two of them were male (73.3%). Mean age is 25 ± 3.89 years (mean $\pm$ SD). The results of APTT, PT, plasminogen, antiplasmin, fibrinopeptide A, t-PA and plasminogen activator activity (PA activity) are shown in table 1. All values were within normal range (see Appendix II). Tests for FDPs were done in all with negative results. All samples were collected in the morning (from 9:00 AM to 11:30 AM). The correlation coefficients of various values are listed in table 2. Fibrinolytic parameters were not correlated with age. Only significant correlation coefficients are between APTT and PT and between plasminogen and antiplasmin activities. There was no difference between sex in laboratory values (table 3).

Patient Group (32 cases)

Fourteen of them were male (43.75%). Mean age was 31.28 ± 14.13 years (mean $\pm$ SD), ranging from 15 to 75 years. Bitten sites are shown in figure 1. Snake species could be identified as *Trimeresurus albolabris* in 18 cases (56.25%), *Trimeresurus macrops* in 7 cases (21.88%) and the others were unidentified.

Table 1 Parameters in Control group

Parameter	Mean	S.D.
Age (years)	25.00	3.59
APTT (sec)	28.96	2.06
PT (sec)	12.08	0.72
Plasminogen (%)	114.18	12.39
Antiplasmin (%)	106.62	11.36
PA activity (IU/ml)	0.1159	0.0510
t-PA antigen (ng/ml)	4.75	3.71
FpA (ng/ml)	3.59	8.33

Table 2 Correlation coefficient between parameters in control group

	age	APTT	PT	plasminogen	antiplasmin	PA activity	Fp A
APTT	0.0333						
PT	-0.0972	0.5540*					
plasminogen	0.2043	-0.3453	-0.3382				
antiplasmin	0.2422	-0.2272	-0.2646	0.3844*			
PA activity	-0.0900	0.0573	-0.0302	-0.0160	-0.0884		
Fp A	0.0481	-0.0661	0.1073	0.1650	0.3596	0.0289	
t-PA	0.1655	-0.1006	-0.0846	0.3362	0.3030	0.0958	-0.0560

* p value < 0.05

Table 3 Diffrences between Sex in Control Group

Parameter	Male (n=22)	Female (n=8)	p values
	(mean $\pm$ SD)		
age (years)	25.30 $\pm$ 3.51	24.10 $\pm$ 3.91	NS
APTT (sec)	29.28 $\pm$ 2.15	27.94 $\pm$ 1.42	NS
PT (sec)	12.04 $\pm$ 0.75	12.20 $\pm$ 0.64	NS
Plasminogen(%)	112.8 $\pm$ 12.8	117.9 $\pm$ 11.0	NS
Antiplasmin(%)	107.7 $\pm$ 11.8	103.7 $\pm$ 10.2	NS
PA activity (IU/ml)	0.122 $\pm$ 0.052	0.099 $\pm$ 0.046	NS
Fp A (ng/ml)	3.04 $\pm$ 9.17	5.03 $\pm$ 5.81	NS
t-PA (ng/ml)	5.26 $\pm$ 4.21	3.35 $\pm$ 0.91	NS

NS = Not Significant

Figure 1 Bitten Sites

Figure 1 Bitten Sites

- arm 3%
- finger 25%
- hand 25%
- toe 13%
- foot 34%
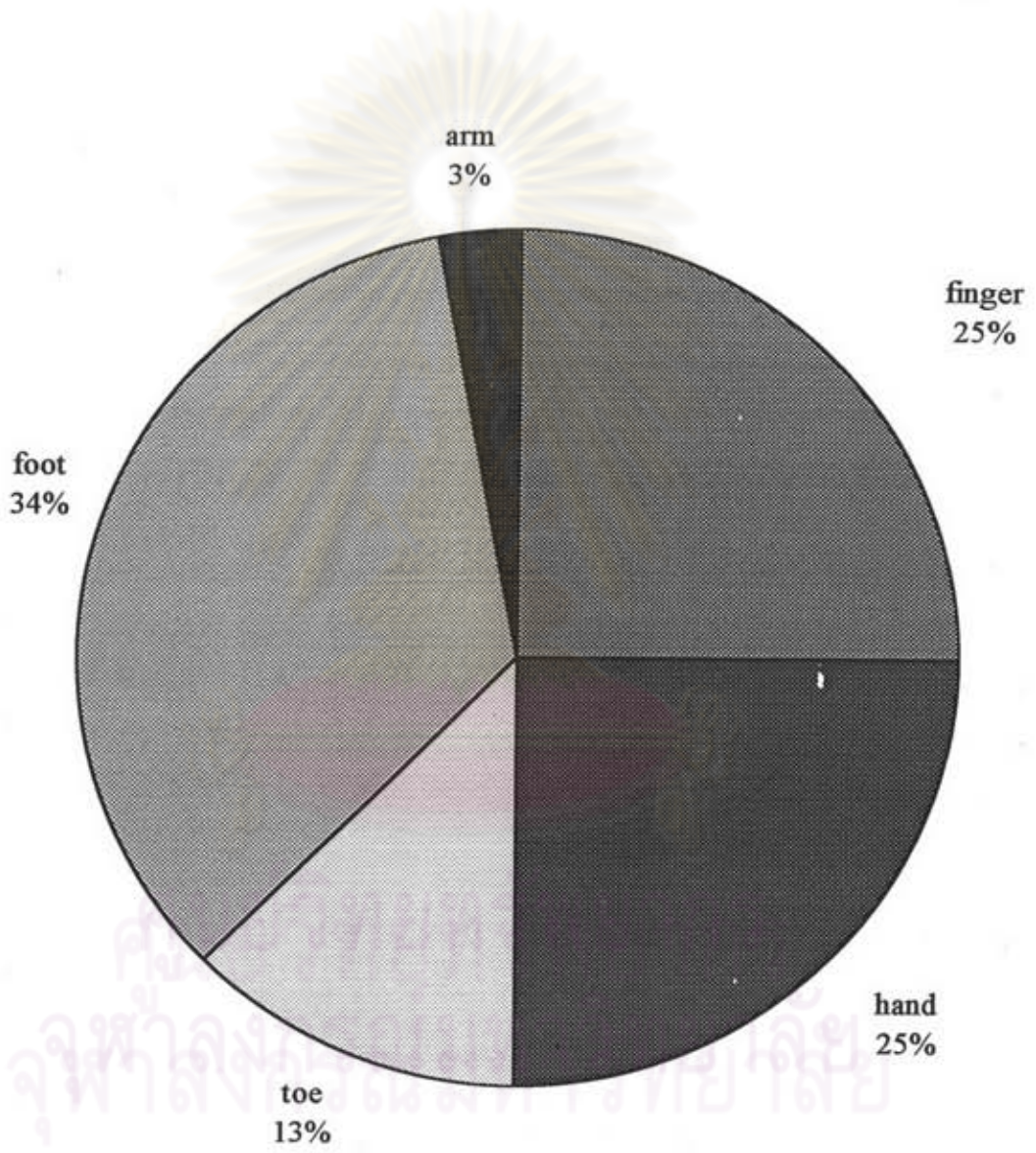
Twenty four samples (75%) were collected in the morning (9:00 AM to 11:30 AM), 5 samples were collected in the afternoon (1:00 PM to 3:30 PM) and one at night (11:30 PM). The time after bites were averagely 31.4 ± 46.5 hours (mean $\pm$ SD), ranging from 1.5 to 264 hours. Mean edema score was 1.67 or across one articulation. Ecchymosis, blister and necrosis were present in 3 (9.38%), 4 (12.5%) and 1 (3.13%) patients respectively. Only one case had systemic bleeding. The other two patients who required antivenin had unclotted VCT. The abnormalities of various values are listed on table 4.

Among screening tests (platelet count, APTT, PT and TT), platelet count was most often abnormal followed by TT, PT and APTT respectively. Performing PT in addition to TT did not increase the positive rate and APTT also did not detect abnormality more than PT. Therefore TT was the single most sensitive test of the three. Hypofibrinogenemia is detected in all cases of TT prolongation.

ELT could not be done in three because of extreme hypofibrinogenemia. With exception of these 3, all hypofibrinogenemic cases had shortened ELT. Plasminogen and/or antiplasmin activities were low in all hypofibrinogenemic cases. Low antiplasmin activity with normal plasminogen activity was found in four cases and one of them was hypofibrinogenemic. Low plasminogen activity with normal antiplasmin activity was found in two cases and one of them had low fibrinogen. Means of these values were shown in table 5.

Fibrinopeptide A were done in only 14 cases and 12 of them (85.7%) had levels more than 50 ng/ml which were beyond the standard curve. Therefore, we could not determine FpA levels exactly. However, we can assume that they were markedly elevated to more than 15 times of control.

Table 4 Abnormal Findings in Case Group

Findings	Number (cases)	Percent
Thrombocytopenia	13	40.6
APTT prolongation	4	13.0
PT prolongation	5	16.2
TT prolongation	6	18.8
Hypofibrinogenemia	9	28.1
Shortened ELT (n=29)	15	51.7
Low plasminogen	11	34.4
Low antiplasmin	13	40.6
Low plasminogen or antiplasmin	15	46.9

Table 5 Means of Parameters in Case Group

Parameters	Mean	S.D.
Age (years)	31.28	14.13
Platelet count ($/mm^3$)	204343.8	111860.7
APTT (sec)	31.63	7.48
PT (sec)	16.56	9.21
TT (sec)	22.10	27.92
Fibrinogen (mg%)	244.7	111.03
FDPs	40.47	31.17
Plasminogen (%)	92.09	29.68
Antiplasmin (%)	83.02	28.52
PA activity (IU/ml)	0.2533	0.2764
t-PA (ng/ml)	8.48	7.05

There was no statistically significant difference of laboratory values between sex and time after bites (table 6 and 7). The differences between two snake species were not statistically significant but *T. macrops* seemed to have less severe changes (table 8).The abnormalities found in 7 cases of *T. macops* bites were thrombocytopenia in 2, shortened ELT in 2 and hypoantiplasminemia in 1. All *T. macrops* bite cases had normal APTT, PT, TT and fibrinogen levels. The only one patient who had undetectable FDP was identified to be *T macrops* bite. Correlation coefficients of these parameters are listed on table 9. Fibrinolytic parameters were not correlated with age of the patients. Platelet counts were correlated with coagulogram and degree of plasminogen and antiplasmin activity depletion. Plasminogen and antiplasmin levels were correlated very strongly to fibrinogen levels. The degree of t-PA elevation was also correlated with coagulopathy but less strongly than plasminogen and antiplasmin. Surprisingly, PA activities were not correlated well with coagulopathy and not correlated with t-PA levels. Comparison between cases with and without fibrinolytic activation (with or without low plasminogen and/or antiplasmin) showed higher t-PA in the group with fibrinolytic activation. Plasminogen activator activities were also higher, but not statistically significant (table 10). Scattergrams showing interrelations between these parameters are figure 2-9.

Comparison between Case and Control Group

Compared with control group, the patients were older. They had prolonged APTT and PT, low plasminogen and antiplasmin. Both plasminogen activator antigen and activity were also higher in case group with statistical significance. Both were about two times above controls. In contrast, fibrinopeptide A levels were markedly elevated at least more than 15 times of controls (table 11). Differences of plasminogen activity, antiplasmin activity, t-PA antigen, plasminogen activator activity and fibrinopeptide levels between case and control group are shown in figure 10 to 14.

Table 6 Differences of Parameters between Sex in Case Group

Parameters	Male (n=14)	Female (n=18)	p values
	(mean $\pm$ SD)		
age (years)	30.93 $\pm$ 14.59	31.50$\pm$ 14.19	NS
Platelet count (/mm^3)	215643 $\pm$ 112690	195556 $\pm$ 113664	NS
APTT (sec)	32.76 $\pm$ 7.95	30.75 $\pm$ 7.19	NS
PT (sec)	16.59 $\pm$ 8.44	16.53 $\pm$ 10.01	NS
TT (sec)	23.34 $\pm$ 30.94	21.13 $\pm$ 26.22	NS
Fibrinogen (mg%)	237.6 $\pm$ 112.6	250.1 $\pm$ 112.7	NS
FDPs (mg/ml)	40.54 $\pm$ 32.37	40.56 $\pm$ 31.15	NS
Plasminogen(%)	93.53 $\pm$ 31.02	90.97 $\pm$ 29.46	NS
Antiplasmin(%)	82.82 $\pm$ 33.08	83.17 $\pm$ 25.42	NS
PA activity (IU/ml)	0.193 $\pm$ 0.194	0.300 $\pm$ 0.324	NS
t-PA (ng/ml)	9.14 $\pm$ 9.45	7.97 $\pm$ 4.65	NS

NS = Not Significant

Table 7 Differences of Parameters between Early and Late Specimen Collections in Case Group

| Parameters | Presentation | | p values |
| | less than 24 hrs (n=19) | more than 24 hrs (n=13) | |
	(mean $\pm$ SD)		
Platelet count (/mm^3)	210895 $\pm$ 124350	194769 $\pm$ 94673	NS
APTT (sec)	32.31 $\pm$ 7.46	30.63 $\pm$ 7.68	NS
PT (sec)	16.58 $\pm$ 7.54	16.52 $\pm$ 11.58	NS
TT (sec)	22.71 $\pm$ 27.44	21.2 $\pm$ 29.72	NS
Fibrinogen (mg%)	233.8 $\pm$ 106.8	260.5.1 $\pm$ 119.46	NS
FDPs (mg/ml)	41.32 $\pm$ 32.14	39.23 $\pm$ 30.95	NS
Plasminogen(%)	88.49 $\pm$ 34.27	97.35 $\pm$ 21.55	NS
Antiplasmin(%)	82.92 $\pm$ 32.04	83.15 $\pm$ 23.67	NS
PA activity (IU/ml)	0.269 $\pm$ 0.254	0.231 $\pm$ 0.316	NS
t-PA (ng/ml)	8.54 $\pm$ 8.16	8.40 $\pm$ 5.32	NS

NS = Not Significant

Table 8 Differences of Parameters between Two Snake Species in Case Group

Parameters	T. albolabris (n=18)	T. macrops (n=7)	p values
	(mean $\pm$ SD)		
age (years)	32.39 $\pm$ 13.29	36.00 $\pm$ 19.81	NS
time after bites (hrs)	24.2 $\pm$ 23.1	25.2 $\pm$ 15.6	NS
Platelet count (/mm^3)	199222 $\pm$ 106642	292571 $\pm$ 101618	NS
APTT (sec)	33.04 $\pm$ 9.23	28.87 $\pm$ 2.33	NS
PT (sec)	17.82 $\pm$ 11.95	14.11 $\pm$ 1.88	NS
TT (sec)	26.96 $\pm$ 35.90	13.16 $\pm$ 1.88	NS
Fibrinogen (mg%)	246.9 $\pm$ 126.3	301.0 $\pm$ 69.8	NS
FDPs (mg/ml)	38.61 $\pm$ 31.52	37.14 $\pm$ 33.02	NS
Plasminogen(%)	93.42 $\pm$ 31.25	109.14 $\pm$ 10.42	NS
Antiplasmin(%)	84.33 $\pm$ 32.95	96.64 $\pm$ 12.63	NS
PA activity (IU/ml)	0.267 $\pm$ 0.307	0.154 $\pm$ 0.146	NS
t-PA (ng/ml)	9.90 $\pm$ 9.00	6.70 $\pm$ 3.17	NS

NS = Not Significant

Table 9 Correlation Coefficients between Parameters in Case Group

	plt	APTT	PT	TT	fibrino	FDP	Plg	Antipl	PA	t-PA
age	-.191	-.104	-.006	-.019	.318	.006	.277	.052	-.031	.105
plt		-.420*	-.472$^+$	-.423*	.481$^+$	-.564$^+$	.623$^+$	+.699$^+$	-.242	-.281
APTT			.950$^+$	.950$^+$	-.751$^+$	.432*	-.681$^+$	-.618$^+$	.174	.516$^+$
PT				.962$^+$	-.702$^+$	.479$^+$	-.666$^+$	-.622$^+$	.251	.434*
TT					-.719$^+$	.466$^+$	-.676$^+$	-.650$^+$	.245	.611$^+$
fibrino						-.544$^+$	.843$^+$	.710$^+$	-.323	-.423*
FDP							-.544$^+$	-.677$^+$	.374*	.331
Plg								.829$^+$	-.188	-.411*
Antipl									-.245	-.578$^+$
PA										.219

plt = platelet count

fibrino = fibrinogen

Plg = Plasminogen

Antipl = Antiplasmin

PA = Plasminogen Activator Activity

t-PA = tissue type Plasminogen activator antigen

* = $p < 0.05$

+ = $p < 0.01$

Table 10 Difference of Parameters in Green Pit Viper Bite Patients with and without fibrinolytic activation

Parameters	Fibrinolytic Activation		p values
	yes (n=16)	no (n=16)	
	(mean $\pm$ SD)		
age (years)	32.19 $\pm$ 13.14	30.38$\pm$ 15.44	NS
time after bites (hrs)	41.7 $\pm$ 61.8	21.2 $\pm$ 20.6	NS
Platelet count (/mm^3)	131000 $\pm$ 78053	277688 $\pm$ 91059	0.000032
APTT (sec)	23.86 $\pm$ 10.01	29.39 $\pm$ 2.13	NS
PT (sec)	20.00 $\pm$ 12.20	13.11 $\pm$ 1.04	0.03
TT (sec)	32.15 $\pm$ 37.33	12.04 $\pm$ 1.63	0.04
Fibrinogen (mg%)	187.94 $\pm$ 127.33	301.4 $\pm$ 49.01	< 0.00001
FDPs (mg/ml)	56.25 $\pm$ 29.18	24.69 $\pm$ 25.00	0.002596
Plasminogen(%)	73.62 $\pm$ 31.28	110.56 $\pm$ 10.98	0.000104
Antiplasmin(%)	60.09 $\pm$ 20.21	105.94 $\pm$ 12.29	< 0.00001
PA activity (IU/ml)	0.344 $\pm$ 0.322	0.163 $\pm$ 0.192	0.0619
t-PA (ng/ml)	10.95 $\pm$ 9.30	6.02 $\pm$ 1.77	0.0459

NS = Not Significant

Table 11 Difference between Cases and Controls

Parameters	Case	Control	p values
	(mean values)		
Age (years)	31.28	25.00	0.0213
APTT (sec)	31.63	28.96	0.0638
PT (sec)	16.56	12.08	0.0101
Plasminogen (%)	92.09	114.18	0.0004
Antiplasmin (%)	83.02	106.62	0.0001
PA activity (IU/ml)	0.2533	0.1159	0.0095
t-PA antigen (ng/ml)	8.48	4.75	0.0123

Figure 2 Correlation between Fibrinogen and Plasminogen/ Antiplasmin
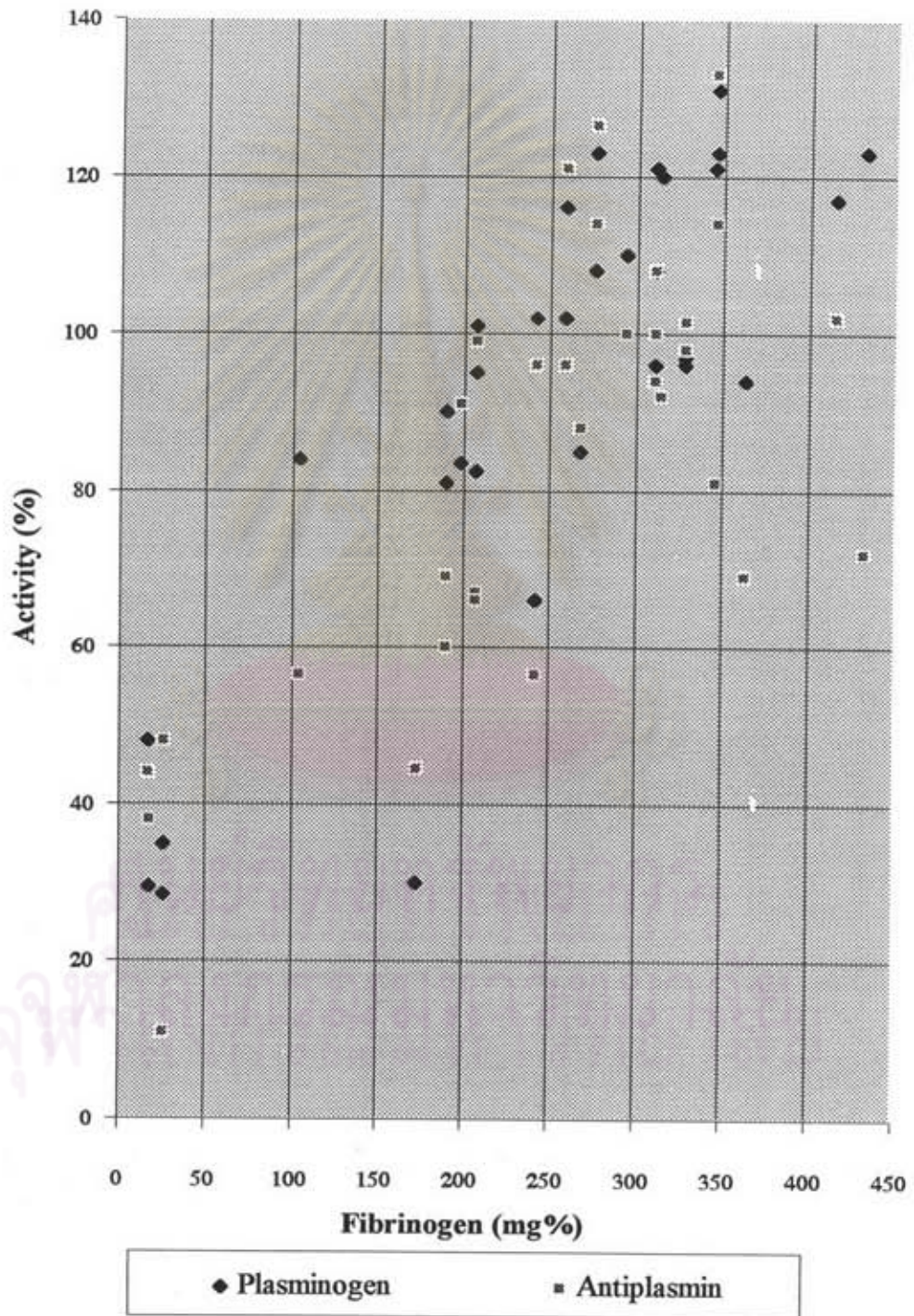
Figure 3 Correlation between FDPs and Plasminogen/

Antiplasmin

FDPs (mg/ml)
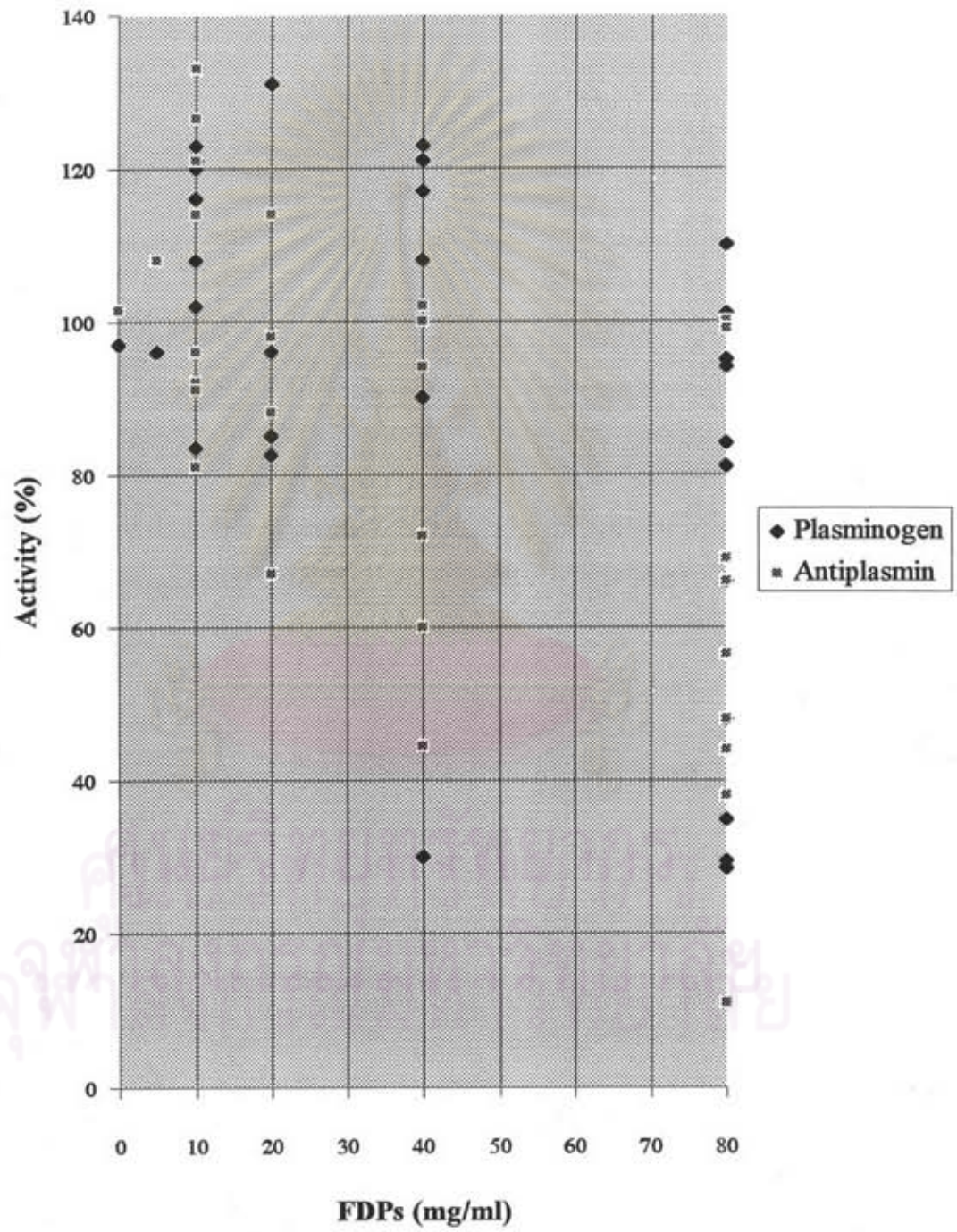
Figure 4 Correlation between t-PA and Fibrinogen
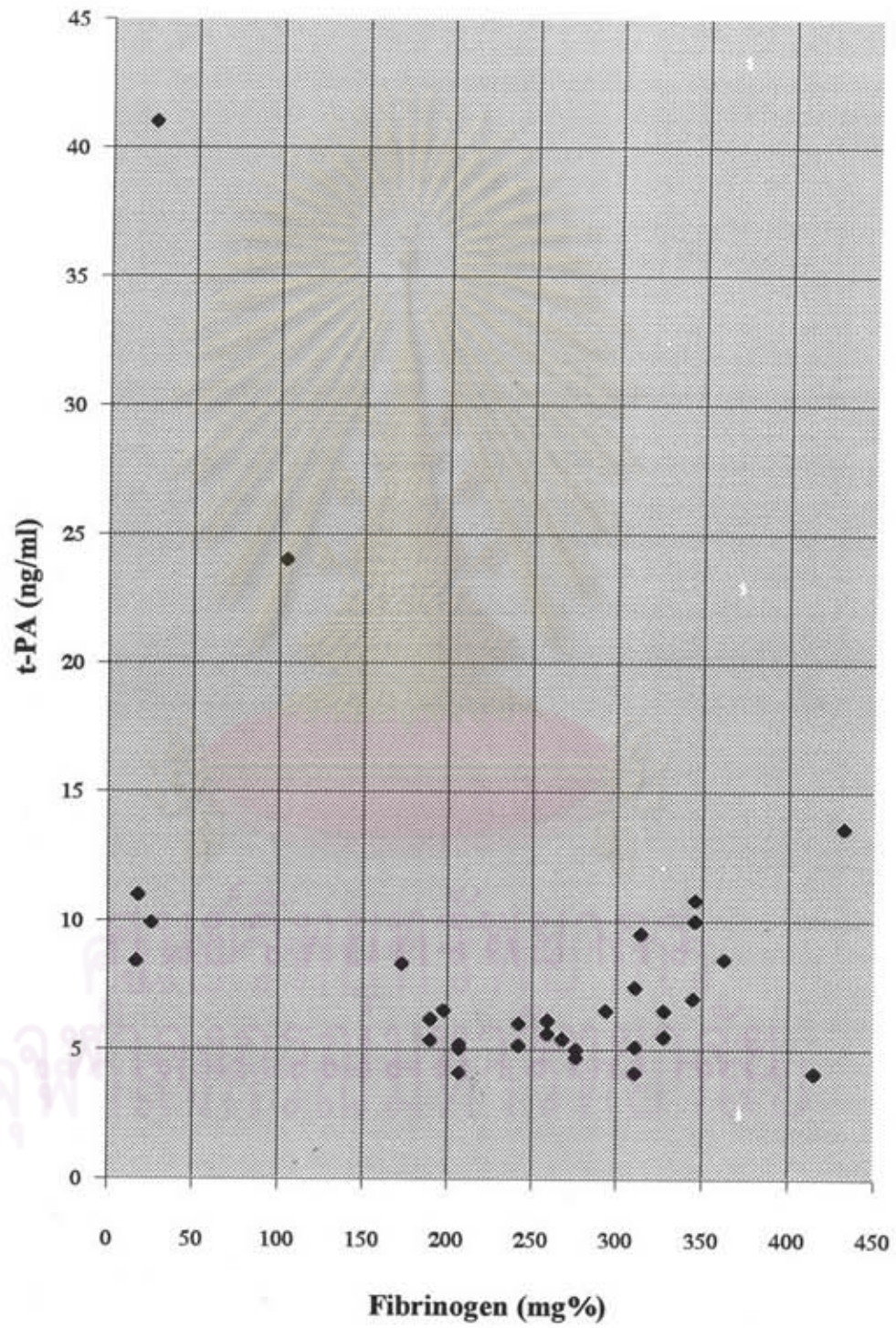
Fibrinogen (mg%)

Figure 5 Correlation between FDPs and t-PA
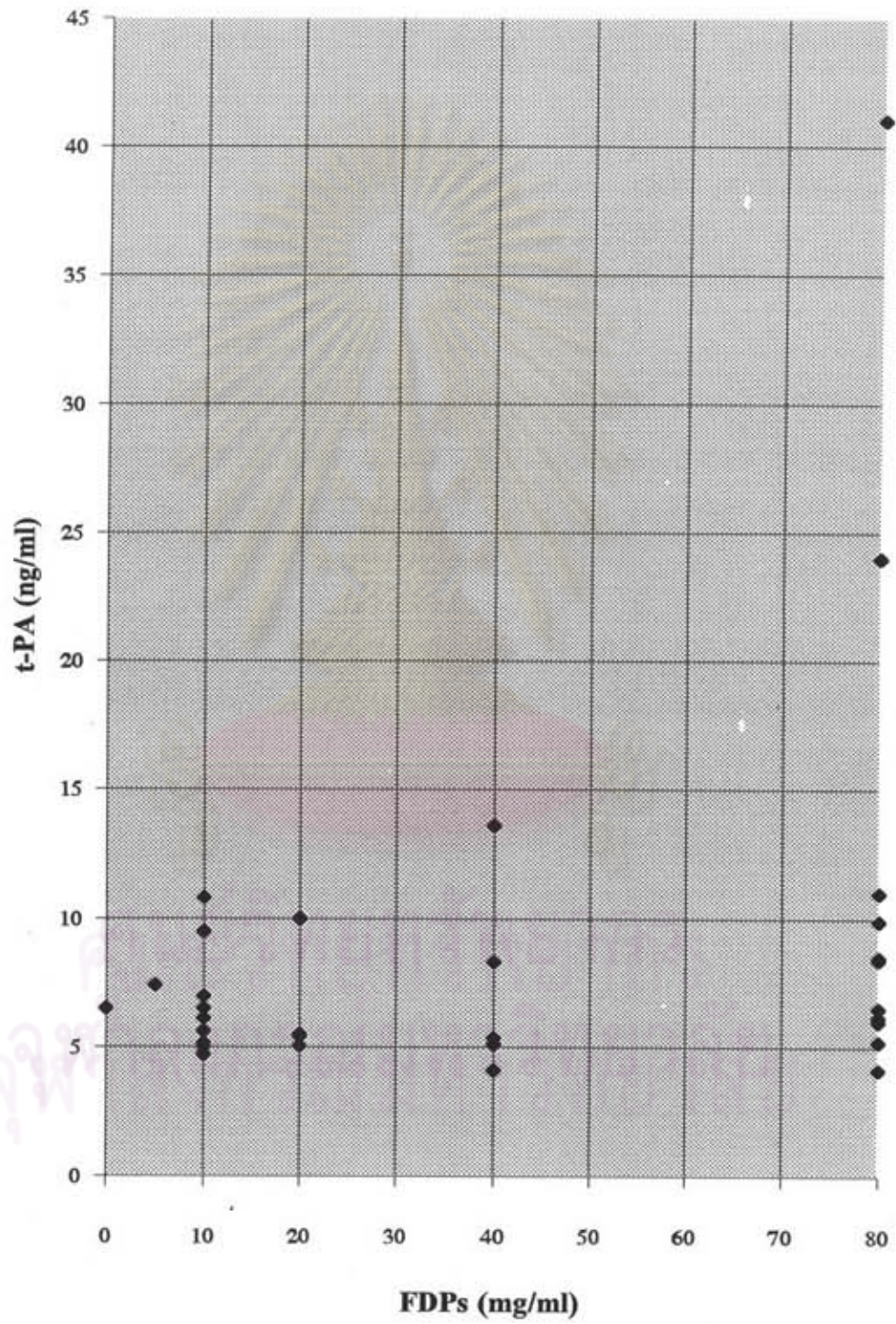
Figure 6 Correlation between Fibrinogen and PA activity
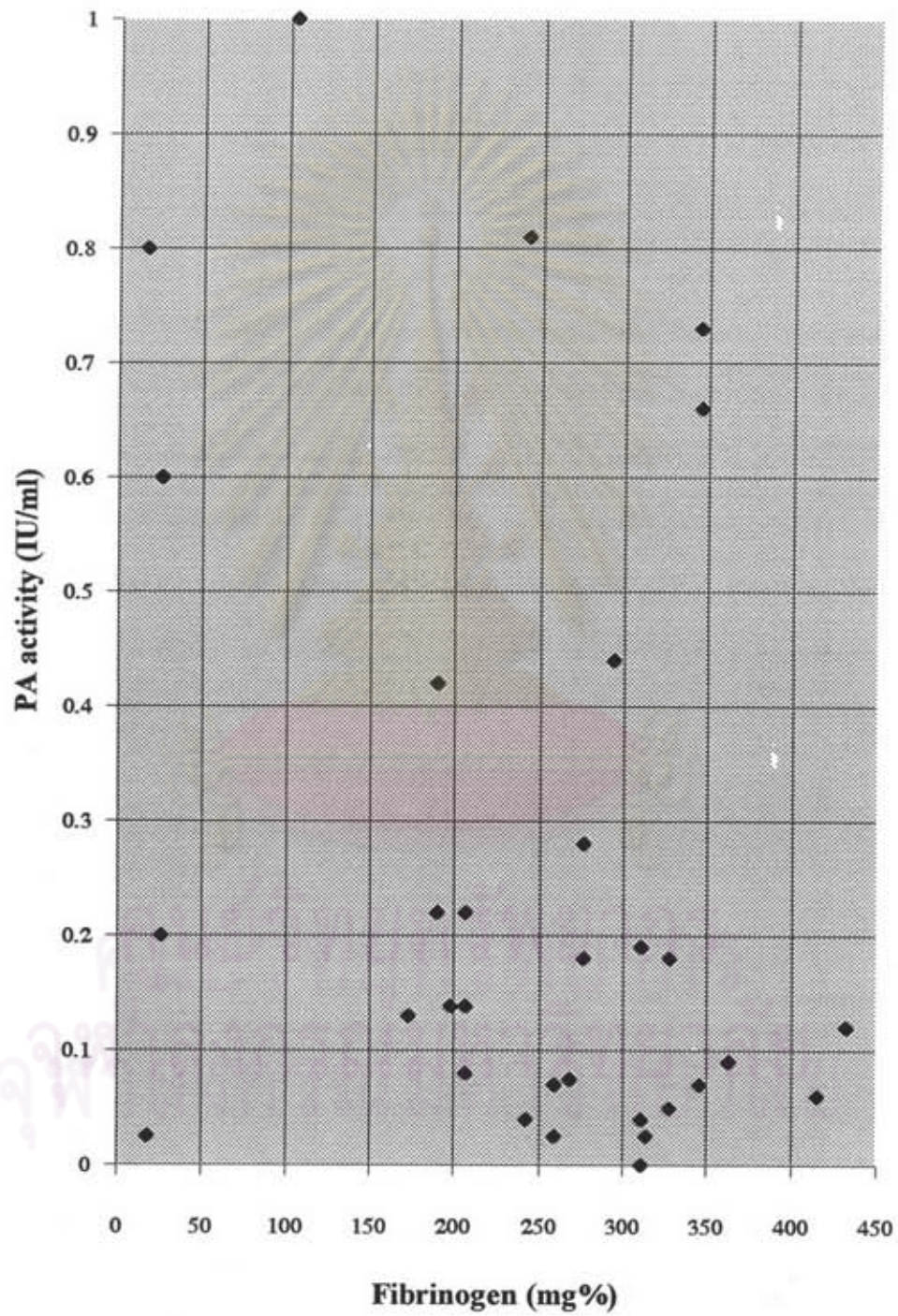
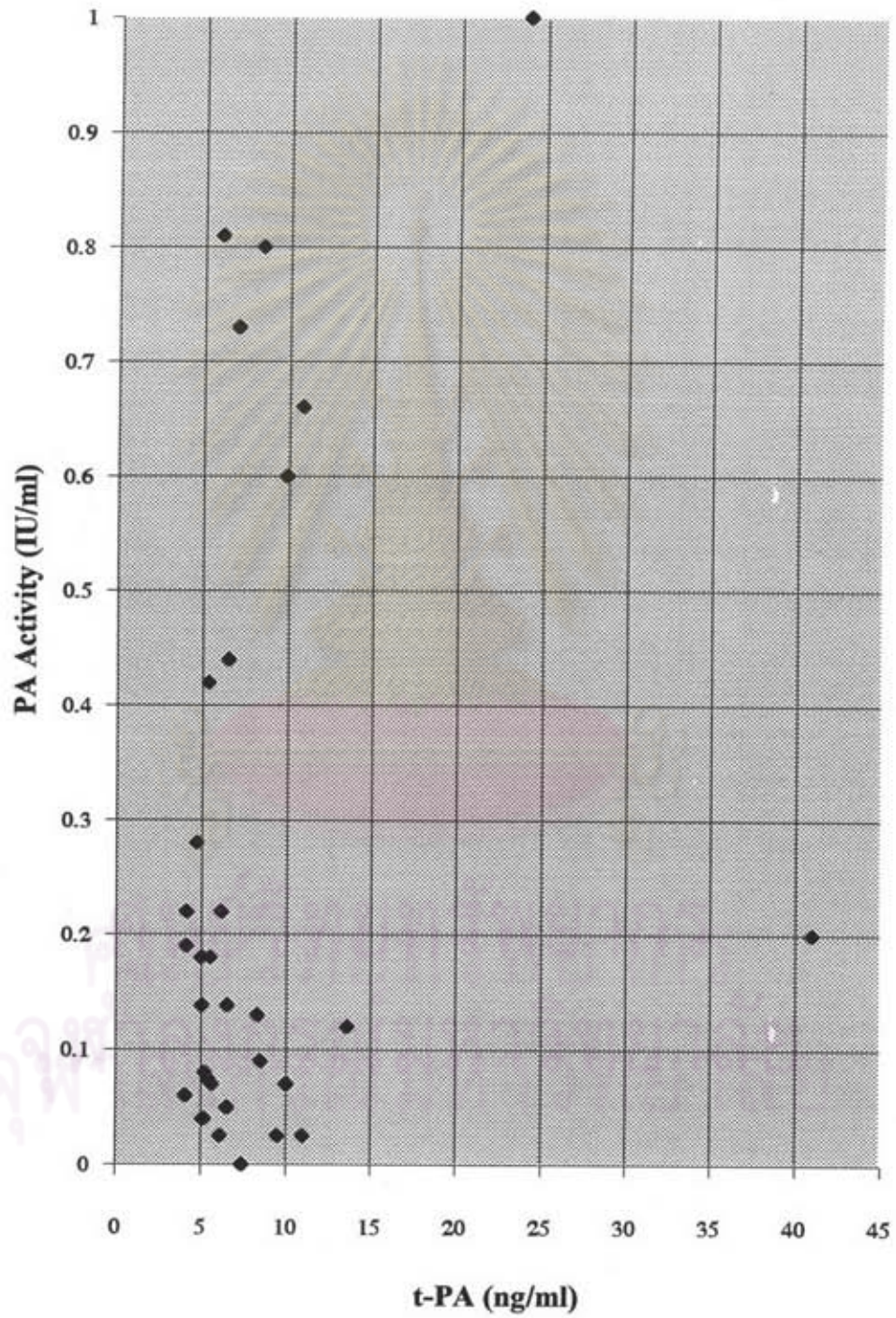Figure 7 Correlation between FDPs Level and PA Activity
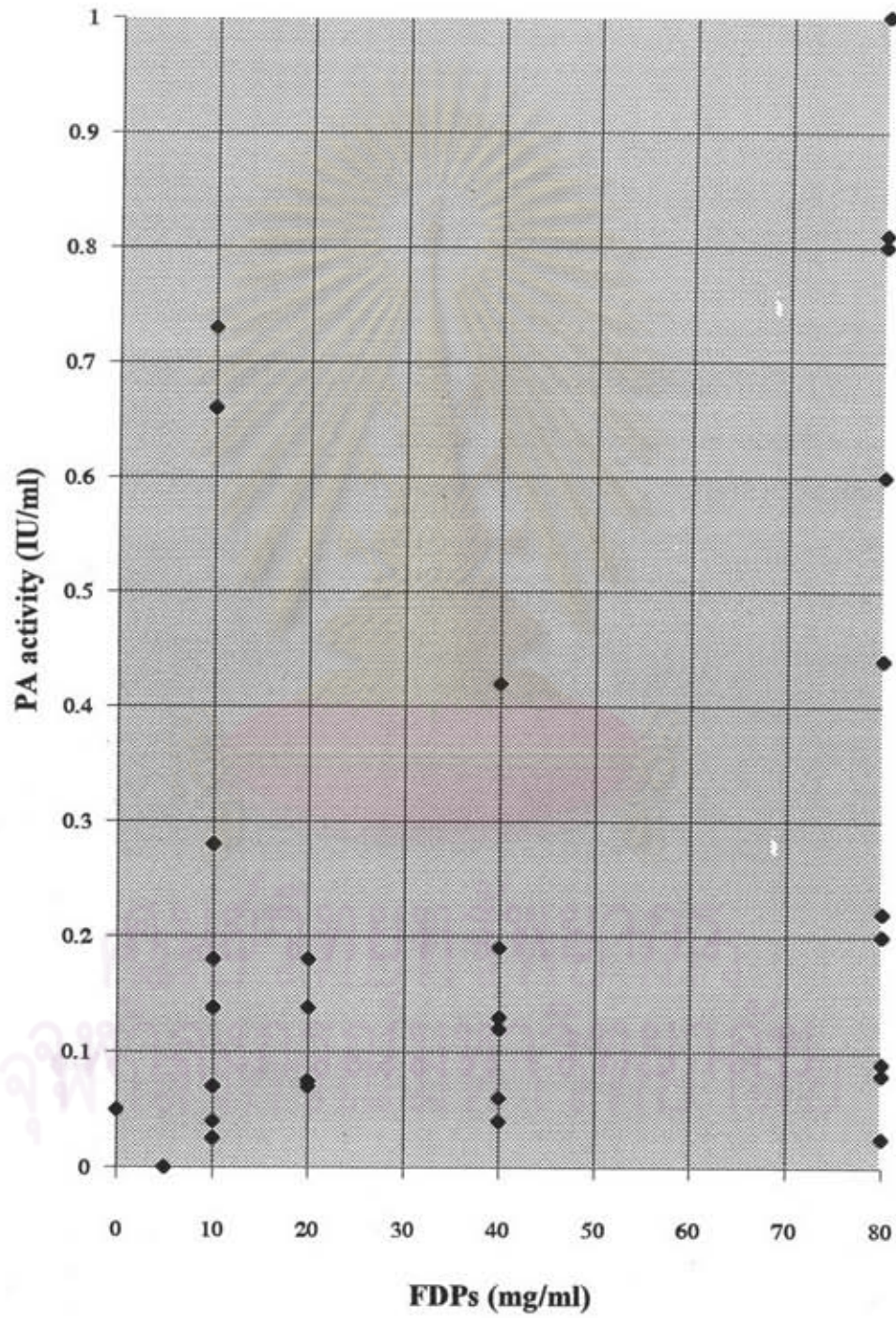
Figure 8 Correlation between t-PA Antigen and PA Activity

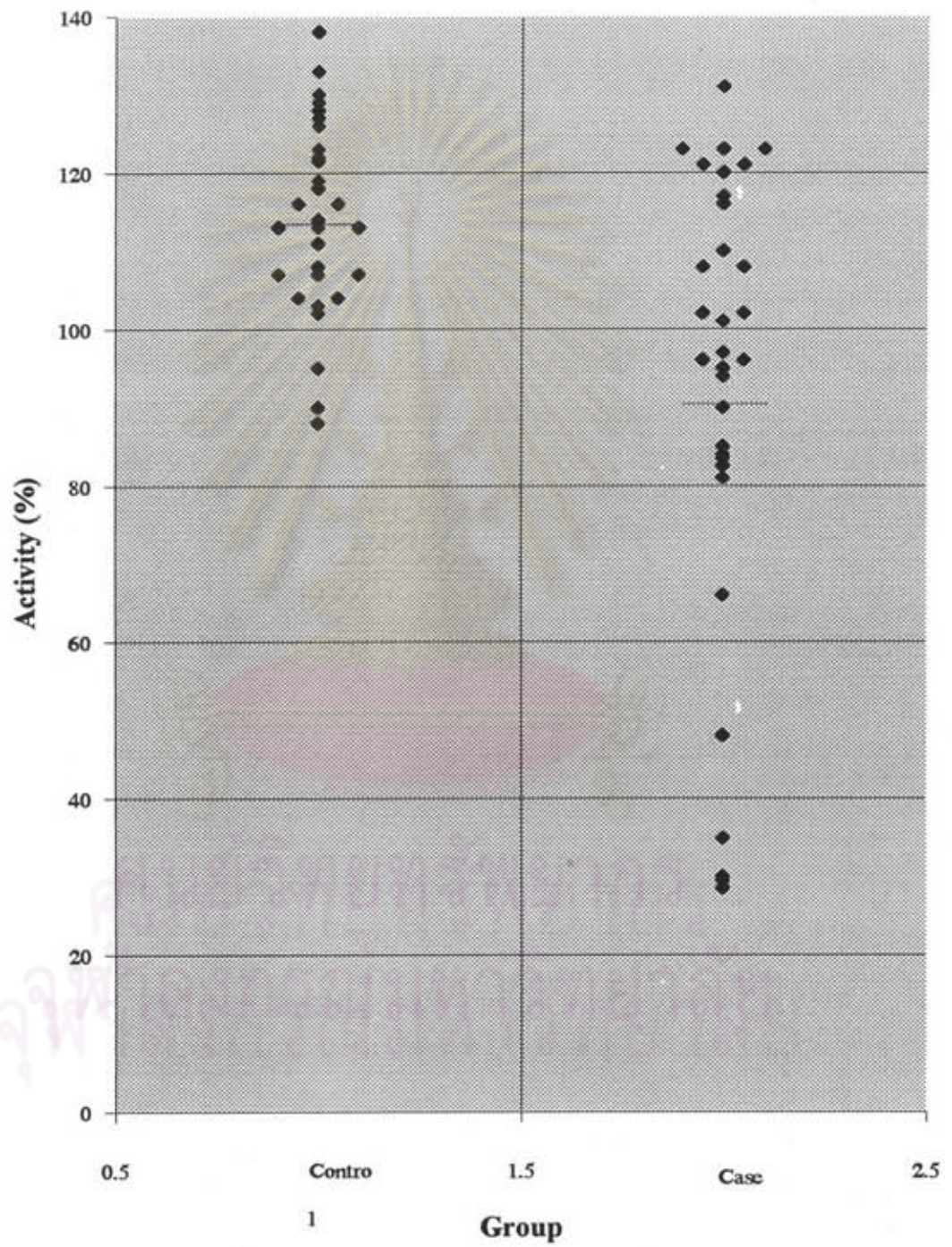**Figure 9 Correlation between Platelet Count and Fibrinogen Level**
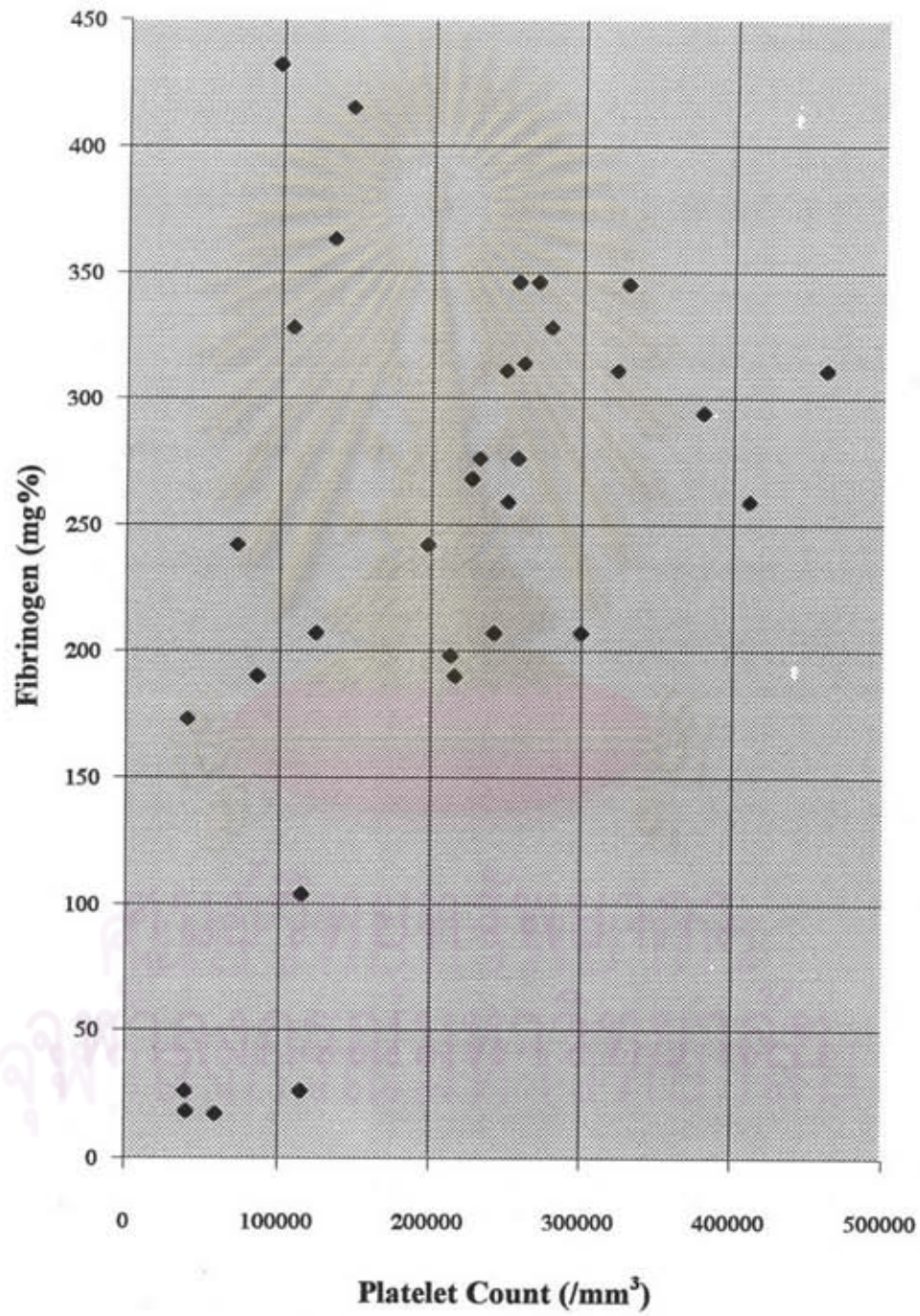
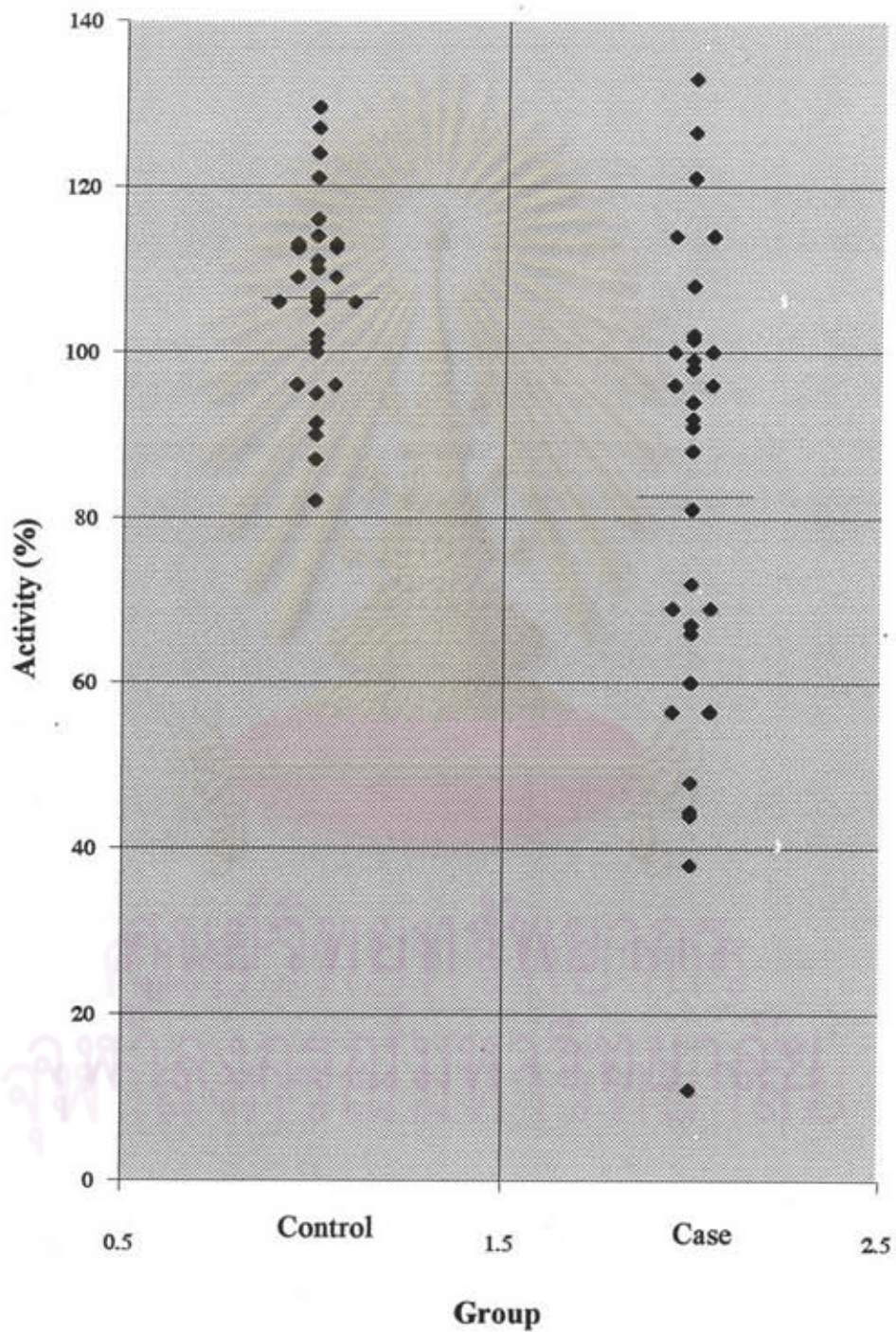**Figure 10 Plasminogen Levels in Case and Control Group**
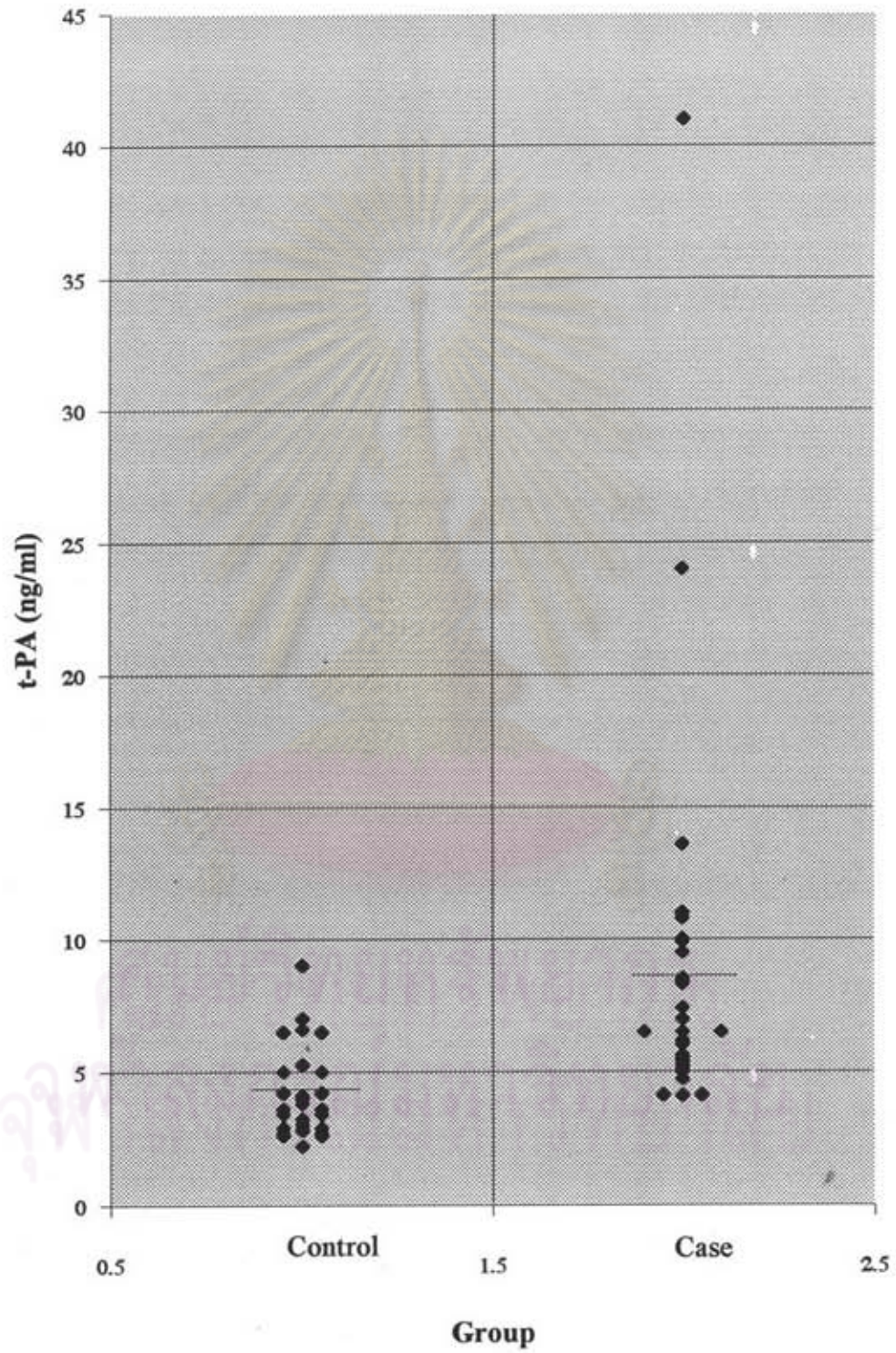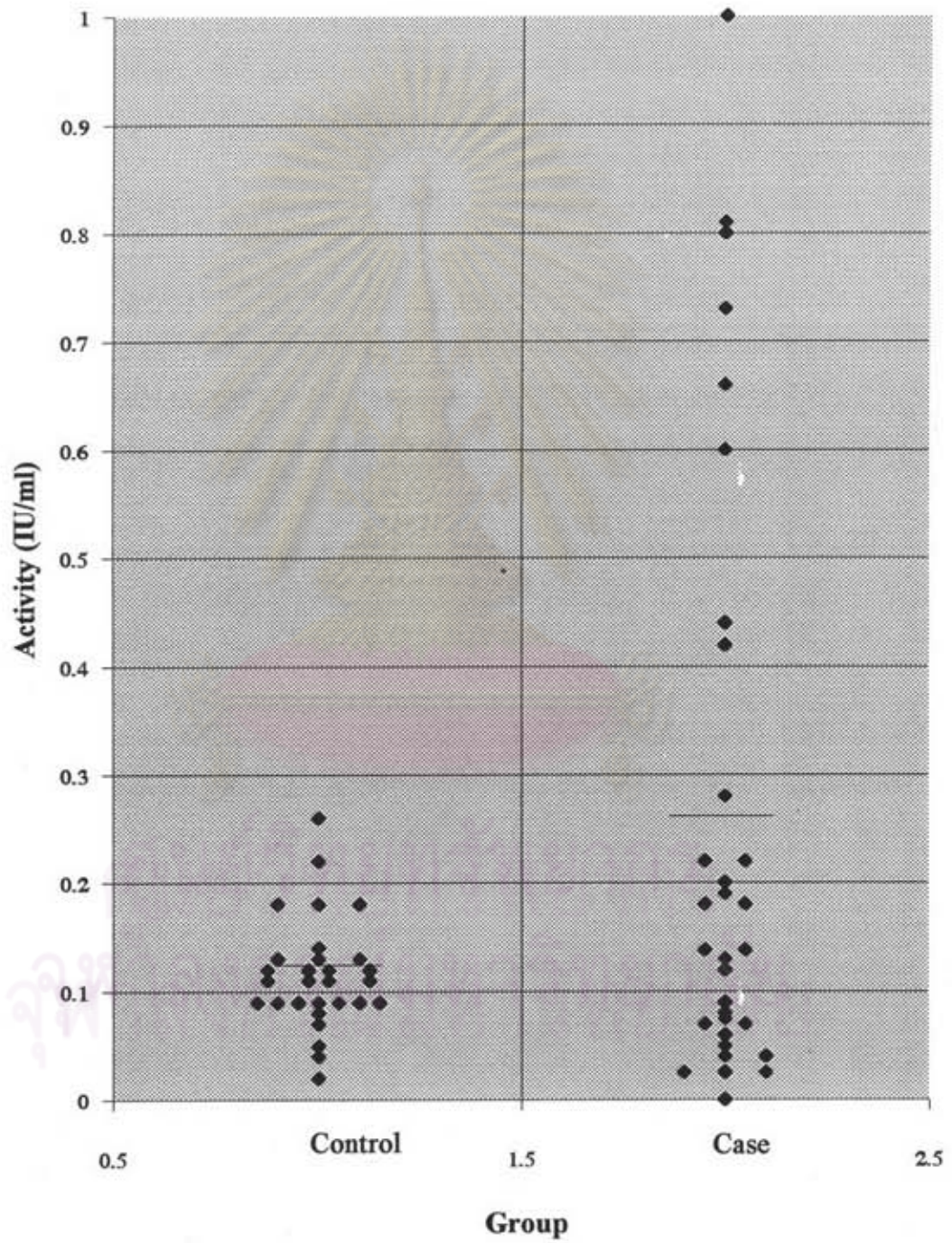
Figure 11 Antiplasmin Activity in Case and Control Group

Figure 12 t-PA Antigen Levels in Case and Control Group

Group

Figure 13 Plasminogen Activator Activity in Case and Control Group

Figure 14 Fibrinopeptide A Levels in Case and Control Group

Because of the diurnal variation of fibrinolytic parameters, we try excluding the specimens collected in the afternoon and at night. After reanalysis, plasminogen activator activity and antigen were still higer than control group (table 12). Female controls (n=8) and female cases (n=18) had t-PA levels $3.35 \pm .910$ ng/ml and 7.79 ± 4.65 ng/ml respectively the difference of which is statistically significant (p=0.01). Their plasma PA activities were 0.099 ± 0.046 and 0.300 ± 0.324 IU/ml respectively (p=0.0967). For male subjects, t-PA levels and PA activities were 5.26 ± 4.21 ng/ml and 0.122 ± 0.052 IU/ml for control and 9.14 ± 9.45 ng/ml and 0.193 ± 0.194 IU/ml respectively. Values of cases were higher than controls but not significantly different.

Response to Antivenin

In 3 cases, follow-up parameters could be done every 6 hours after antivenin until VCT became normal. VCT and fibrinopeptide A levels declined in all three after antivenin. Plasminogen and antiplasmin activities also increased promptly. However, plasminogen activator antigen and activity decreased in some but not all cases. Paradoxically, increasing plasminogen activator activity after antivenin was observed in one patient.

Table 12 Difference between Cases and Controls (Only in Cases with Morning Sample Collection)

Parameters	Case (n=26) (mean ± SD)	Control (n=30)	p values
age (years)	32.42 ± 15.42	25.00± 3.59	0.0313
APTT (sec)	31.23 ± 6.59	28.96 ± 2.06	0.0790
PT (sec)	16.25 ± 9.16	12.08 ± 0.72	0.0160
Plasminogen(%)	91.55 ± 29.42	114.18 ± 12.39	0.0003
Antiplasmin(%)	83.60 ± 27.65	106.62 ± 11.36	0.0001
PA activity (IU/ml)	0.245 ± 0.291	0.116 ± 0.051	0.0203
t-PA (ng/ml)	7.63 ± 4.07	4.75 ± 3.71	0.0076