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# The role of platelets in the coagulopathy of heatstroke – a study of platelet aggregation in heatstroke patients during the pilgrimage (Haj) to Makkah

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**Platelet aggregation was undertaken in platelet rich plasma in 34 heat-stroke patients during the Muslim pilgrimage (Haj) to Makkah; 18 were males and 16 were females; their ages ranged from 36 to 80 years (mean ± SD = 58 ± 10). Platelet aggregability, on arrival at the Heatstroke Centres, was markedly inhibited in response to adrenaline, collagen, arachidonic acid and ristocetin but not to ADP. Responses to decreasing ADP doses (20.0, 2.0, 1.0 and 0.5 μmol/l) showed hyperaggregability in 12 patients, inhibited responses in 16 and normal responses in 6 patients. Aggregation responses were not significantly different when comparing patients with bleeding manifestations ($n$ = 10), with those without bleeding ($n$ = 24). Haemostatic parameters including plasma fibrinogen, ATIII and platelet count, were markedly reduced in the two patient groups who showed hyperaggrebable and depressed aggregation responses, but not in those with normal responses. These results lead us to conclude that: (1) platelet activation is a frequent feature of heatstroke; (2) in heatstroke altered aggregation responses, whether hyperaggregable or depressed, occur simultaneously with a consumption coagulopathy.**

## Introduction

Many recent studies have attempted to document the haemostatic defect that accompanies the severe form of heatstroke, which is often characterized by widespread bleeding manifestations.[1–5] Evidence from these and other studies indicates that a consumption coagulopathy is a common feature of heatstroke whether or not it is accompanied by a bleeding tendency. This conclusion is based mainly on the recording of diminished levels of a wide variety of clotting and fibrinolytic factors, including platelets.[1,2] In addition, in an effort to identify the trigger to the coagulopathy of heatstroke we found *in vitro* evidence indicating that heat directly activates platelets.[6] If such an activation process occurs in a similar manner *in vivo*, it will lead to secondary activation of the coagulation system and may result in a consumption coagulopathy.

The potential role played by platelets in heatstroke has recently been reviewed[7] and it would seem that platelet function has never been systematically studied in heatstroke patients. For this reason, and as a continuation of our earlier experimental observations, the current study was aimed at investigating platelet function as measured by platelet aggregation responses. These responses were related to both the level of plasma haemostatic parameters as well as the occurrence of bleeding manifestations.

## Materials and methods

This study was conducted during the pilgrimage (Haj) to Makkah in the summer of 1993 when the ambient temperature was 40–46°C. A total of 34 patients were

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studied on their arrival to the Heatstroke Centres in Mina and Jiyad Hospitals, in the Makkah region: 18 patients were males and 16 were females. Their ages ranged from 36 to 80 years old (mean + SD = 58 ± 10) and their mean rectal temperature was 42.0 ± 0.8°C (range 40–43.5). Ten patients had bleeding manifestations[1] ranging from prolonged bleeding from venepuncture sites and petechial haemorrhages, to more severe bleeding from multiple sites such as gums, conjunctivae, haematuria and gastrointestinal bleeding (classical severe disseminated intravascular coagulation (DIC) syndrome). All patients fulfilled the diagnostic criteria of heatstroke: rectal temperature of 40°C and above, dry, hot, flushed skin and disturbances of consciousness varying from drowsiness to deep coma. Efforts were made to find out whether the patients were on any form of medication, but it was not possible to obtain reliable information on this point.

All patients were tested on their arrival to the Heatstroke Centre (sample A), after establishing the diagnosis of heatstroke and before any therapeutic intervention. Also, a second blood sample was collected 6 h later (sample B) after the patient had undergone the standard management procedures of cooling and hydration. Fresh frozen plasma and platelet concentrate were given to bleeders, when indicated.

Blood samples were collected in sodium citrate (0.11 mmol/l) in a blood:citrate ratio of 9:1 and were processed immediately as previously described.[8] Platelet aggregation was undertaken in platelet rich plasma (PRP) in response to ADP (20.0, 2.0, 1.0, 0.5 µmol/l), adrenaline (100 µmol/l), collagen (0.19 g/l), arachidonic acid (1.64 mmol/l) and ristocetin (1.5 mg/ml), as detailed elsewhere.[8] Platelet counts were recorded from the routine haemogram which is usually performed on the patients' arrival. The methods employed in the coagulation screening tests, as well as individual factor assays (plasma fibrinogen, ATIII, plasminogen, alpha 2-antiplasmin, protein C activity and free protein S activity) are described elsewhere.[1]

### *Statistical methods*

The results were analysed using the Mann–Whitney U-test. One-way ANOVA was conducted using program 3S from the BMDP Statistical Package to compare the group means for each haemostatic parameter. Multiple comparison tests were carried out using the Kruscal–Wallis test.

## Results

### *The effect of heat stroke on platelet aggregation*

Data on platelet aggregation responses in heatstroke patients on arrival for treatment were compared with our Coagulation Laboratory reference values obtained from a healthy population.[9] There was a statistically significant reduction in the aggregation responses in heatstroke patients and this is most noticeable in the responses to arachidonic acid, adrenaline, collagen and ristocetin, while responses to ADP were least affected (Table 1).

### *Platelet aggregation in response to decreasing doses of ADP*

Since dose–response aggregometry reflects the degree of platelet activation more accurately than single dose studies,[6,10] the results of responses to decreasing doses of ADP in heatstroke patients were analysed and the results obtained were as follows: normal responses ($n$ = 6), hyperaggrebable ($n$ = 12) and depressed ($n$ = 16). It was noted that on most occasions (8 out of 12) hyperaggregability to ADP was obtained in individuals

**Table 1.** Platelet aggregation values in heatstroke patients versus healthy controls (local reference values). Results are expressed as the slope (S) and maximum aggregation (%), of the aggregation curves

| Agonist | Controls mean ± SD ($n$) | Patients mean ± SD ($n$) | $P$ value |
|---|---|---|---|
| ADP 20.0 (S) | 35 ± 10 (167) | 36 ± 19 (34) | 0.72 |
| ADP 20.0 (%) | 56 ± 17 (167) | 60 ± 23 (34) | 0.27 |
| ADP 2.0 (S) | 31 ± 10 (545) | 29 ± 14 (34) | 0.20 |
| ADP 2.0 (%) | 50 ± 22 (545) | 49 ± 22 (34) | 0.86 |
| ADP 1.0 (S) | 25 ± 9 (225) | 24 ± 9 (34) | 0.32 |
| ADP 1.0 (%) | 33 ± 20 (225) | 35 ± 21 (34) | 0.71 |
| ADP 0.5 (S) | Not done | 15 ± 5 (34) | – |
| ADP 0.5 (%) | Not done | 21 ± 15 (34) | – |
| Adrenaline (S) | 12 ± 9 (511) | 10 ± 5 (34) | 0.27 |
| Adrenaline (%) | 50 ± 29 (511) | 32 ± 22 (34) | 0.003* |
| Arachidonic acid (S) | 30 ± 14 (371) | 15 ± 12 (34) | < 0.001* |
| Arachidonic acid (%) | 65 ± 20 (371) | 36 ± 23 (34) | < 0.001* |
| Collagen (S) | 37 ± 11 (462) | 20 ± 15 (34) | < 0.001* |
| Collagen (%) | 65 ± 12 (462) | 48 ± 29 (34) | < 0.001* |
| Ristocetin (S) | 37 ± 15 (403) | 29 ± 20 (34) | 0.004* |
| Ristocetin (%) | 78 ± 14 (403) | 57 ± 28 (34) | 0.001* |

*Significant at the 5% level of significance

Table 2. Platelet aggregation responses in heatstroke patients: a comparison between bleeders versus non-bleeders. Results are expressed as mean ± standard deviation

| Agonist | Bleeders (*n*) | Non-bleeders (*n*) |
|---|---|---|
| ADP 20.0 (S) | 43 ± 14 (9) | 33 ± 19 (18)* |
| ADP 20.0 (%) | 74 ± 11 (9) | 54 ± 23 (18)* |
| ADP 2.0 (S) | 31 ± 11 (10) | 29 ± 15 (21) |
| ADP 2.0 (%) | 52 ± 16 (10) | 48 ± 24 (22) |
| ADP 1.0 (S) | 24 ± 9 (10) | 23 ± 9 (18) |
| ADP 1.0 (%) | 37 ± 22 (10) | 33 ± 20 (18) |
| ADP 0.5 (S) | 15 ± 7 (7) | 14 ± 4 (11) |
| ADP 0.5 (%) | 19 ± 14 (7) | 22 ± 15 (11) |
| Adrenaline (S) | 11 ± 4 (9) | 9 ± 5 (24) |
| Adrenaline (%) | 36 ± 21 (9) | 30 ± 22 (24) |
| Arachidonic acid (S) | 16 ± 9 (10) | 15 ± 13 (23) |
| Arachidonic acid (%) | 41 ± 17 (10) | 34 ± 24 (23) |
| Collagen (S) | 21 ± 13 (10) | 10 ± 15 (23) |
| Collagen (%) | 55 ± 132 (9) | 46 ± 27 (23) |
| Ristocetin (S) | 27 ± 17 (9) | 30 ± 20 (21) |
| Ristocetin (%) | 54 ± 25 (9) | 58 ± 28 (21) |

*Significant at the 5% level of significance

displaying depressed responses to other agonists especially arachidonic acid, adrenaline and collagen.

*Follow-up observations of aggregation responses in heatstroke patients*

No significant differences were noted when the aggregation responses obtained on arrival (sample A) were compared to those obtained 6 h after the management procedures had commenced (sample B); detailed results are not shown.

*Platelet aggregation in bleeders versus non-bleeders*

There was a tendency for the aggregation response to be higher in bleeders than in non-bleeders but the differences attained statistical significance only for the responses to ADP (Table 2).

*Relationship between the aggregation responses and the levels of plasma haemostatic parameters*

When comparing the haemostatic factors in patients displaying 'depressed' and 'hyperaggregable' responses to those with 'normal' aggregation responses, the differences reached statistical significance only for fibrinogen, α-2-antiplasmin and proteins C and S (Table 3), as follows:

*Fibrinogen.* The mean fibrinogen levels in three patient groups differed significantly ($P = 0.0379$; ANOVA). Also, the difference between the mean fibrinogen for the 'normal' versus the 'hyperaggregable' group was statistically significant ($P < 0.05$).

*Alpha-2-antiplasmin.* The mean value for the three groups of patients differed significantly ($P = 0.0117$; ANOVA). The difference between the mean α-2-antiplasmin levels for the 'normal' versus the 'hyperaggregable' group was also statistically significant ($P < 0.05$).

*Protein C.* The mean value for the three groups of patients differed significantly ($P = 0.0032$; ANOVA). Besides, the differences in the mean protein C concentration in patients with 'normal' aggregation responses versus those with 'hyperaggregable' as well as the 'depressed' responses were statistically significant ($P < 0.05$, for both).

*Protein S.* The mean protein S concentration in the three groups of patients differed significantly ($P = 0.0045$; ANOVA). The difference between the mean protein S concentrations in patients with 'normals' as compared with the levels in those with 'depressed' responses was statistically significant ($P < 0.05$).

## Discussion

Many studies have confirmed the association between heatstroke and the consumption of coagulation and fibrinolytic factors.[1-5] The present study is a further

Table 3. Haemostatic parameters in heatstroke patients: a comparison between those displaying hyperaggregability or depressed aggregation responses versus those with normal responses

| Haemostatic parameters | Normals mean ± SD (*n*) | Hyperaggregable responses mean ± SD (*n*) | Depressed responses mean ± SD (*n*) |
|---|---|---|---|
| Prothrombin time (%) | 128.2 ± 34.2 (6) | 123.8 ± 20.3 (12) | 136.4 ± 25.5 (14) |
| Partial thromboplastin time (%) | 123.8 ± 57.4 (6) | 126.9 ± 40.0 (12) | 142.5 ± 175.9 (14) |
| Thrombin time (%) | 107.8 ± 29.4 (6) | 106.0 ± 18.4 (11) | 111.5 ± 23.8 (12) |
| Reptilase time (%) | 102.0 ± 20.1 (6) | 128.4 ± 30.5 (12) | 134.0 ± 52.9 (13) |
| Fibrinogen (mg/dl) | 338.2 ± 35.0 (6) | 258.6 ± 75.2* (12) | 277.7 ± 107.1* (13) |
| Antithrombin III (%) | 70.2 ± 15.3 (6) | 67.3 ± 16.8 (12) | 65.5 ± 19.5 (14) |
| Plasminogen (%) | 92.5 ± 14.1 (6) | 82.2 ± 18.1 (12) | 79.6 ± 15.6 (14) |
| α-2-antiplasmin (%) | 90.3 ± 9.0 (6) | 62.5 ± 16.8* (12) | 70.9 ± 19.5 (14) |
| Protein C (%) | 96.3 ± 13.5 (6) | 55.3 ± 20.6* (12) | 61.5 ± 17.3* (14) |
| Protein S (%) | 93.3 ± 11.6 (6) | 86.7 ± 32.9 (11) | 54.8 ± 20.0* (12) |
| Platelets × $10^9$/l | 218.5 ± 69.1 (6) | 177.4 ± 90.0 (11) | 163.2 ± 135.3 (14) |

*Mean was significantly different from that of controls at the 5% level of significance

attempt to characterize the haemostatic defect(s) that accompany heatstroke, especially the role of platelets. Earlier evidence implicated platelets in the trigger of the coagulopathy of heatstroke[1-6] and consumption of platelets in heatstroke patients has been reported in many publications (reviewed recently[7]). Such reduction of platelet numbers can be the result of peripheral consumption rather than diminished production, since bone marrow examination in heatstroke patients showed no evidence of megakaryocyte damage.[2] Thrombocytopenia of heatstroke was mostly, but not always, accompanied by haemorrhagic complications.[1,2,4,5]

In support of our earlier *in vitro* observations,[6] the present study attempted to find evidence of *in vivo* platelet activation in heatstroke patients. Initial analysis of the results (Table 1) showed that heatstroke patients exhibited the combination of enhanced responses to ADP and inhibited platelet responses to collagen, arachidonic acid and adrenaline. This pattern is similar to that obtained when heating platelets *in vitro*.[6]

These findings prompted us to seek evidence of platelet activation employing the responses to decreasing doses of ADP.[6] Platelets from 12 out of 34 patients, showed hyperaggregability while responses were normal in six and depressed in 16. One explanation for these results is that those patients were tested at various stages of the platelet activation process;[11,12] platelets in one group of patients ($n$ = 6) were unactivated, and their responses remained normal; in a second group ($n$ = 12) platelets were activated and hyperresponsive, while in a third group ($n$ = 16) platelets were at the end of the activation process and became refractory even to ADP. Alternatively, this wide spectrum of platelet aggregation responses can also be explained by the fact that these patients were tested at various time intervals after exposure to heat. This explanation is in line with our *in vitro* observations,[6] that once platelets are directly activated by heat, they progressively pass through a phase of hyperaggregation. If left unchecked the platelets will eventually lose their responses to agonists.

The spectrum of abnormal aggregation responses in heatstroke patients differs markedly from the responses seen in healthy individuals,[9] suggesting that despite the wide variability in platelet aggregability among individuals, platelet activity in heatstroke patients undergoes extensive alteration as a result of the body exposure to heat. At least, the prevalence of hyperaggregability is sufficient direct evidence that *in vivo* platelet activation is a prime feature in heatstroke patients.

These changes in platelet function cannot prevail in isolation from simultaneous changes in other haemostatic factors. Platelet counts showed marked reduction in patients who displayed hyperaggregable responses as well as those with depressed responses, but counts were much higher (but not normal) in those patients with normal aggregation responses. This suggests that a platelet consumption process is already underway in heatstroke patients, being more severe in those who displayed marked alterations in the platelet aggregation responses, whether hyperaggregation or depressed responses.

A concurrent diminution was also noted in other haemostatic factors especially the known markers of consumption coagulopathy, namely fibrinogen as well as the natural anticoagulants AT III, proteins C and S. In the absence of laboratory evidence of liver disease in the studied patients, the reduction in the levels of these natural anticoagulants, especially proteins C and S can be taken as another feature of consumption coagulopathy[13] in heatstroke patients. It is therefore possible that the *in vivo* consumption of various components of the haemostatic system is closely related to platelet activation, which perhaps takes a leading (triggering) role in this dynamic process.

In most patients it was not possible to obtain accurate information regarding medication in the period before admission. Thus, the inhibitory effect of drug therapy on platelet aggregation responses could not be ruled out. However, earlier observations have shown that platelets can still be activated by heat even in the presence of aspirin,[6,14] the activation process occurring via a pathway, other than the known ADP and prostaglandin pathways of platelet activation, the so-called membrane modulation.[14]

It has recently been stated that temperature is an under-appreciated problem in the genesis of coagulopathy and that platelet function is critically temperature-dependent.[15] Although these revelations stem from observations in hypothermia,[15] it is becoming increasingly clear, after the recent observation in heatstroke and cancer patients exposed to therapeutic hyperthermia,[16] that exposure of platelets to extremes of temperature has a detrimental effect on the haemostatic system, i.e. it can trigger a consumptive coagulopathy.

There is no evidence yet that plasmatic haemostatic factors can be activated by exposure to such extremes of temperature (whether hypo- or hyperthermia). Indeed there is evidence to the opposite that plasmatic factors, *in vitro*, are heat labile and tend to lose activity unless frozen.[17] Platelets seem to be an exception.

In conclusion, the results of this study suggest that significant *in vivo* platelet activation is a feature of heatstroke. However, the measurement of platelet aggregation *per se* could not be directly related to the degree of the consumptive coagulopathy or the occurrence of bleeding symptoms in these patients.

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