Investigating the influence of age, gender and ABO blood group on ADAMTS-13 antigen and activity levels in healthy Arabs

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Dear Sir,

It has been reported that approximately 30% of the genetic variations that influence von Willebrand Factor (VWF) levels in plasma are due to the ABO blood group of the individuals. The effect of ABO blood type on VWF expression has, therefore, been the subject of many studies over the years. Plasma VWF levels were reported to be significantly lower in group O individuals than in non-O individuals, which correlates with the increased risk of bleeding of the former. Explanations for the reduced levels of VWF in group O individuals range from the effect of ABO blood group on the rate of synthesis/secretion of VWF, to an effect on the survival of the protein and its clearance from plasma.

The VWF cleaving protease ADAMTS-13, (the 13th member of the ADAMTS family of metalloproteases characterized by the combination of a disintegrin-like and metalloprotease with thrombospondins type 1 motif), was found to dispose of VWF physiologically by cleaving the peptide bond between tyrosine and methionine in the central A2 domain of VWF. The gene for ADAMTS-13 is located on chromosome 9q approximately 140,000 nucleotides from the ABO locus; this close proximity may also play a role in the 30% genetic variation that ABO exerts on VWF levels[1].

Maintaining a balance between VWF and ADAMTS-13 is crucial for blood haemostasis. While many pathological conditions are associated with an imbalance between these two proteins, several physiological factors have also been found to play a role in affecting the expression of these proteins.

In this study we aimed to determine the effects of age, gender and ABO phenotype on the activity and antigenic levels of ADAMTS-13 in healthy males and females of Arab ethnicity. A hypothesis that the lower levels of VWF in group O individuals are mirrored by higher levels of ADAMTS-13 was also tested in this study.

After obtaining consent, venous blood was collected into vacuum collection tubes containing sodium citrate (3.8 %, w/v)-(Becton, Dickinson and Company, New Jersey, USA), from 200 apparently healthy subjects (100 males and 100 females). All subjects were non-smokers and were undergoing a routine check-up at the time of blood collection. Standard, commercially available, enzyme-linked immunosorbent assay (ELISA) kits were used to determine levels of the studied protein according to the manufacturer's description (Technoclone, Vienna, Austria).

In order to measure VWF antigen levels, we used a sandwich ELISA with co-incubation of VWF and a secondary conjugated antibody (anti-VWF-POX) in a single step. The ADAMTS-13 antigen assay involved adding first ADAMTS-13 and then, after a washing step, a conjugate working solution containing anti- ADAMTS-13 POX. For the ADAMTS-13 activity assay, a recombinant VWF fragment was immobilised onto an ELISA plate, which encodes the A2 domain and the ADAMTS-13 cleavage site at Tyr1605-Met1606 and is tagged with S-transferase (GST)-histidine (GST-VWF73-His). After adding plasma, the residual, cleaved VWF fragment is measured by using a second monoclonal antibody [horseradish peroxidase (HRP)-conjugated monoclonal anti-N10] that recognises only the cleaved VWF fragment. The chromogenic substrate tetramethylbenzidine (TMB) was used to detect the reaction in all the assays.

Since race was not a factor in this study, as all subjects were of Arab ethnicity, we focused on the effects of age, gender and ABO blood group on VWF and ADAMTS-13 levels. A non-parametric Spearman's correlation analysis was performed to investigate the effects of age on the investigated proteins. As previously reported[2], higher levels of VWF were found with older age ($r=0.269$, $p<0.001$). Given the size of the cohort, it is difficult to determine the degree to which the level changes with increasing age.

Our analysis also showed that ADAMTS-13 activity decreased with age ($r=-0.257$, $p<0.001$), while ADAMTS-13 antigen levels were not affected by increasing age. It is not clear why there is this discrepancy, but the absolute difference between the two proteins appears to be small and is not likely to be of any physiological or clinical relevance (Figure 1). Whether the higher VWF antigen levels in older individuals is a consequence of lower activity of ADAMTS-13 is subject for further analysis.

In order to determine whether gender had an influence on the findings, we compared VWF and ADAMTS-13 levels in males and females, regardless of blood group type. After controlling for age, females had significantly lower levels of VWF ($p<0.001$) compared

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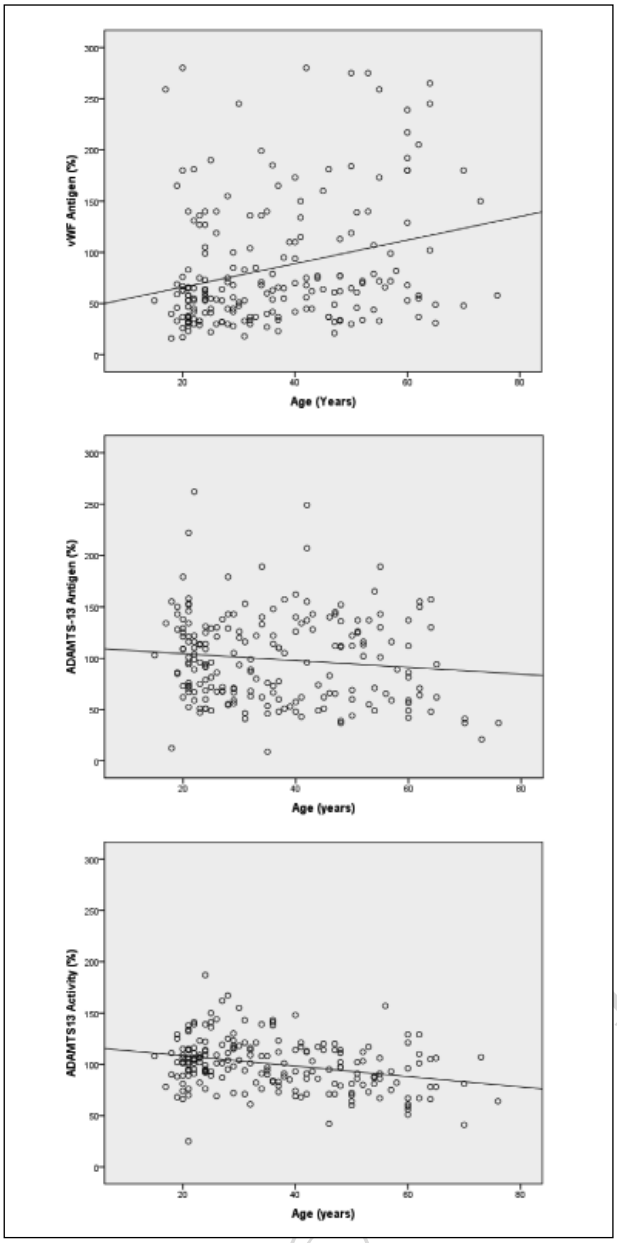

Figure 1 - VWF antigen levels increase with age (r = 0.269), while ADAMTS-13 activity levels decrease (r=−0.257) (p<0.001). ADAMTS-13 antigen levels are not affected by age (p>0.05).

to those in males (Table I). We also found that females had higher levels of ADAMTS-13 antigen (p<0.001); the combination of the results for VWF and ADAMTS-13 antigen levels could indicate that females are more prone to bleeding, but further investigation is recommended in a larger cohort to support or refute this hypothesis.

Eighty-one subjects (40.5 %) in our population had the O blood group and their median age was 32 years (range, 18-70 years), while 119 (59.5 %) were non O-blood group and had a median age of 33 years (range, 15-76). There was not a statistical difference in the age between the two group (p>0.05).

While subjects with O blood group had significantly lower VWF antigen levels than those with non-O blood groups (p=0.003), there were no differences in ADAMTS-13 antigen and activity levels between the two groups (Table I). ADAMTS-13 levels continued to be not different when individual groups were compared (using the Kruskal-Wallis test), but VWF was significantly different (p=0.001) with levels increasing in the following order: O<A<B<AB (results not shown).

After incorporating gender into the ABO blood group analysis, we found that only group O females had significantly lower VWF levels than non-O females [45% (16-275) *vs* 59% (18-181), p<0.01]. It was surprising not to find a difference in VWF levels between group-O and non-O males in our population [85% (21-275) *vs* 85% (23-280), p>0.05]. The lack of a difference may be related to ethnicity. It is well documented that ethnicity plays an important role in determining VWF levels[3]. To our knowledge, no studies on VWF and ABO blood group have previously been conducted in subjects of Arab ethnicity; hence this finding may be unique to our population although a study on a larger population is recommended as the small sample size represent a limitation to the current study.

ADAMTS-13 antigen and activity levels were not different between the two groups in either gender (p>0.05). These findings suggest that whatever is causing lower levels of VWF is not related to quantitative changes in ADAMTS-13 antigen and/or activity but may be more related to structural difference in VWF protein in subjects of different blood groups.

Table I - Comparison of ADAMTS-13 and vWF levels between males and females and between O blood group and non-O blood group subjects. Results are expressed as median (range).

Parameter (Normal Range)	Males (n=100)	Females (n=100)	P value	O blood group (n=81)	Non-O blood group (n=119)	P value
vWF antigen (50-150%)	85 (21-280)	53 (16-275)	<0.001*	53 (16-275)	68 (18-280)	0.003*
ADAMTS-13 Antigen (70-130%)	67 (13-249)	120 (9-262)	<0.001*	109 (9-262)	93.5 (21-249)	0.286
ADAMTS-13 Activity (40-130%)	95 (25-187)	102 (42-143)	0.429	102 (41-144)	101 (25-187)	0.51

*Statistically significant difference.

Although it is still not clear how ABO group can influence the proteolysis of VWF, it has been suggested that in group O individuals, the A2 domain (the site of VWF proteolysis by ADAMTS-13) adopts a conformation more permissive for ADAMTS-13 cleavage. A and B antigens were found to protect against VWF proteolysis, while VWF purified from group O blood has been shown to be cleaved faster by ADAMTS-13 protease[2].

ABO(H) sugars were also reported to affect the susceptibility of VWF to ADAMTS13 cleavage. O'Donnell *et al.* reported that a reduction in the number of terminal sugars on N-linked glycan increases the susceptibility of VWF to ADAMTS-13 proteolysis[4]. A study published in 2010 reported that the degree of sialylation modulated by ABO blood group (rather than ABO group itself) is the reason for altered proteolysis of VWF by ADAMTS-13[5].

Here we have presented the first report on the effect of age, gender and ABO on the expression of VWF and ADAMTS-13 in healthy Arabs. VWF levels increased with age, while ADAMTS-13 activity decreased; however, despite being statistically significant, the correlation between age and these two proteins was weak. We confirmed that the levels of VWF antigen are lower in individuals with O blood group, but only in female subjects, who also had higher ADAMTS-13 antigen levels. ADAMTS-13 antigen and activity levels were not affected by ABO blood group. A more detailed analysis in a larger group of subjects is recommended.

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