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High Molecular Weight Kininogen and the risk of myocardial infarction and ischaemic stroke in young women: the RATIO case-control study

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Abstract

Introduction High molecular weight kininogen (HMWK) deficiency is associated with an increased aPTT, but not with a bleeding diathesis. HMWK is a cofactor of the intrinsic coagulation system and as such a pivotal element in the activation of these proteins. Additionally, it is involved in other biologic processes such as inflammation. It is unknown whether HMWK levels affects the risk of myocardial infarction (MI) and ischaemic stroke (IS).

Methods HMWK levels were measured in the RATIO case-control study, which includes young women with MI (N=205), IS (N=175) and 638 healthy controls. Antigen levels of HMWK were measured with a polyclonal ELISA-based assay and expressed as percentage of pooled normal plasma. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated by means of logistic regression as measures of rate ratios.

Results Mean HWMK levels were similar in MI cases and controls (117%) and slightly higher in IS cases (121%). High levels of HWMK (i.e. ≥90th percentile of controls) were associated with an increase in risk of IS ($OR_{adjusted}$ 1.82, 95%CI 1.00-3.29), whereas the risk of MI was not affected ($OR_{adjusted}$1.05, 0.57-1.91). If anything, low levels (≤10th percentile) were associated with a small increase in risk of both MI and IS.

Conclusions High levels of HWMK increase the risk of IS in young women, whereas its role is less clear for MI.

Introduction

Cardiovascular disease is a major cause of morbidity and mortality in high income countries.[1] Arterial thrombosis is a major contributor to this burden; peripheral arterial disease, myocardial infarction (MI) and ischaemic stroke (IS) all occur when a thrombus is lodged in an artery which subsequently reduces the blood flow to downstream tissue. As multicausal cardiovascular diseases, MI and IS share several risk factors. These include, but are not restricted to, classical risk factors such as hypertension, hypercholesterolemia, obesity, smoking and diabetes.[1] Other factors related to primary and secondary haemostasis are also known to be associated with an increase in risk for both MI and IS.[1–6]

An emerging body of evidence suggests that the intrinsic coagulation proteins are involved in the aetiology of cardiovascular diseases. This is in contrast with prior beliefs: despite a relation with the aPTT, deficiencies of coagulation Factor XII and prekallikreine are not related with a bleeding diathesis, whereas coagulation factor XI (FXI) deficiency can result in a mild bleeding phenotype. Therefore, these proteins were regarded as unimportant in haemostasis.[7] Murine and other laboratory studies indicate that these proteins indeed are not involved in regular haemostasis, but are involved in pathological processes leading to thrombosis.[8–12] Clinical studies also suggest that the intrinsic coagulation proteins are involved in the development of human disease.[13–18] Striking results come from studies on FXI deficiency which is relatively common among Ashkenazi Jews. This trait confers a reduced risk of ischaemic stroke and deep venous thrombosis, but not of myocardial infarction.[19–21] A similar pattern is observed in other studies: evidence of an increased state of activation of the intrinsic coagulation proteins was associated with ischaemic stroke, but not myocardial infarction.[22] This contrasts with middle-aged men in whom the same activation markers were associated with both stroke and coronary heart disease.[23]

High molecular weight kininogeen (HMWK) is a cofactor of FXI and prekallikrein; both enzymes are noncovalently bound to HMWK in the circulation through one of the 4 apple domains present in these structurally homologous proteins.[24–27] HMWK also interacts with platelets: it can bind to platelets through the glycoprotein Ib-V-IX receptor complex on platelets, providing a link between primary and secondary haemostasis.[28] Additionally, HWMK may interact with other surfaces such as endothelium and neutrophils, which confirms the pleiotropic role of HWMK in biological systems.[29] The activated intrinsic coagulation system has several actions such as coagulation activation through FXI

activation by FXIIa and the formation of bradykinin through HMWK splicing by kallikrein, which is formed from its precursor prekallikrein by FXIIa.[25,27] Several recent studies indicate that certain circumstances would favor one of these actions. Misfolded proteins can activate FXII which leads to kallikrein formation without activation of coagulation.[30] Also, HMWK can be activated by prekallikrein leading through FXII independent bradykinin formation.[25] Both mechanisms need a negatively charged surface to facilitate protein-protein interactions and HMWK is thought to facilitate this binding, promoting the activation of the intrinsic coagulation system.[27,29,31] These surfaces include well-established activators such as kaolin and glass, but also nucleic acids and polyphosphates released from activated platelets.[31,32] Studies focused on the role of polyphosphates indicate that the size of the negatively charged surface could be dictating the different mechanisms following contact activation.[33]

HMWK is part of this intrinsic coagulation system, but is also involved in other biological systems related to cardiovascular diseases.[34] Cleavage of HMWK by kallikrein leads to the formation of vasoactive substance bradykinin and cleaved kininogen, which are involved in the innate inflammatory response, blood pressure control, brain edema, the renin-angiotensin system and endothelial cell differentiation.[35–38] HMWK has been sparsely studied in a clinical setting. It was found to be 10% higher in patients suffering from venous thrombosis than in blood donors, suggesting that it is associated with an increased clotting potential.[14] The relation with myocardial infarction was also studied; two small studies showed that HMWK levels were lower in patients than healthy controls.[39,40] However, sample collection in these small studies occurred within hours after the disease onset. Since HWMK levels may be affected by the acute phase these results cannot be easily interpreted.

Previous results from the RATIO study showed that increased levels of activated protein-inhibitor complexes are associated with ischaemic stroke but not myocardial infarction.[22] Of the antigen levels of these proteins, only high levels of FXI were clearly associated with an increase in ischaemic stroke. This study is designed to determined whether HMWK, as co-factor for the activation steps in this system, is also linked to the risk of myocardial infarction and ischaemic stroke in young women.

Material and Methods

Study design & participants The RATIO study is a population-based case-control study, originally set up to investigate the association between the use of oral contraceptives and incident arterial thrombosis. Details of this study have been published before.[2,41,42] In short, women under the age of 50 years who where diagnosed using standard criteria with myocardial infarction, ischaemic stroke or peripheral arterial disease in one of the 16 participating centers were eligible to participate. Healthy controls, frequency-matched on age (5 years categories), area of residence and index date, were contacted via random digit dialing. This analysis focuses on myocardial infarction and ischaemic stroke. During the first phase of RATIO we included 248 cases with myocardial infarction, 203 cases with ischaemic stroke and 925 frequency-matched controls. During the second phase of the study, in which all participants were recontacted to provide either a blood sample or buccal swabs for DNA extraction. Ultimately, blood samples of 205 myocardial infarction cases, 175 ischaemic stroke cases and 638 healthy controls were available. The study was approved by institutional review committees of the participating hospitals and all subjects gave informed consent.

Measurements HMWK levels were measured with a sandwich ELISA based assay using a polyclonal, commercially available antibody kit optimised to reduce signal to noise ratio (CEDARLANCE inc., Burlington, Ontario, Canada). This kit uses a purified coating antibody

Table 1. Characteristics of participants

		Myocardial infarction N=205		Ischaemic stroke N=175		Control N=638	
Mean age		43	(6.1)	39	(7.9)	39	(7.9)
Caucasian ethnicity		195	(95%)	168	(95%)	602	(94%)
History of *							
Hypertension (N, %)		53	(26%)	50	(29%)	40	(6%)
Diabetes (N, %)		10	(5%)	7	(4%)	10	(2%)
Hypercholesterolaemia (N,		21	(10%)	14	(8%)	19	(3%)
Oral contraceptives use (N, %)		81	(40%)	92	(53%)	213	(33%)
Smoking (N, %) *		169	(82%)	105	(60%)	270	(42%)
HMWK †	Mean (SD)	117%	(21%)	121%	(25%)	117%	(22%)
	Median (Q1-Q3)	115%	(103% -131%)	120%	(102%-140%)	115%	(103%-131%)

Abbreviations: HMWK = High molecular weight kininogen, SD = standard deviation, Q1 = 1st quartile, Q3 = 3rd quartile. in the year prior to event (cases) or index year(controls). † levels are expressed as percentage of HMWK levels in normal pooled plasma percentages might not add due to rounding.*

Table 2. Levels of HMWK in relation to baseline characteristics in the control group

HMWK levels in control group*	
10th percentile	92%
25th percentile	103%
50th percentile (median)	115%
75th percentile	131%
90th percentile	145%

Risk factor	HMWK levels* Mean (SD)	difference (95% CI)
Hypertension -	117% (21%)	[ref]
Hypertension +	118% (20%)	1 %(-6% to 8%)
Diabetes -	117% (21%)	[ref]
Diabetes +	131% (23%)	14% (1% to 27%)
Hypercholesterolaemia-	117% (21%)	[ref]
Hypercholesterolaemia+	126% (21%)	8% (-1% to 18%)
Oral contraceptive use -	118% (22%)	[ref]
Oral contraceptive use +	116% (19%)	-2% (-6% to 1%)
Smoking -	118% (20%)	[ref]
Smoking +	116% (21%)	-2% (-6% to 2%)
Age†		0.4% (0.2% to 0.6%)

Abbreviations: HMWK = High molecular weight kininogen, SD = standard deviation, 95% CI = 95% confidence interval. levels are expressed as percentage of HMWK levels in normal pooled plasma. Percentages might not add due to rounding.† difference is expressed in the increase of HMWK levels per year.*

targeted against HMWK, incubated overnight at 2-8° C (CL20027K-C), in combination with a purified peroxidase labeled detection antibody (CL20027K-D). Being an o-phenylenediamine based antibody kit, we measured light absorbance at 490 NM and signal strengths were converted to HMWK levels expressed as percentage of normal pooled plasma. Data on medical history, oral contraceptive use and other patient characteristics were obtained by questionnaire and reflect the year prior to the event (for the cases) or the frequency-matched index date (controls) unless stated otherwise.

Statistical analyses Odds Ratios (ORs) as measures of rate ratios and corresponding 95% confidence intervals (95%CIs) were calculated by means of logistic regression; all models included age (continuous), area of residence and index date (categorical) to account for the

frequency-matching procedure. Traditional risk factors, i.e. hypertension, diabetes, smoking and hypercholesterolaemia were additionally included in subsequent models as putative confounders. To determine the risk associated with extreme levels of HWMK the 10th and 90th percentile of the control group were used as cutoff values for low and high levels (i.e. ≤p10 & ≥p90). Quartile analyses, with categories based on the 25th, 50th and 75th percentile of controls were used to investigate possible dose response associations.

Results

As expected, traditional risk factors such as hypertension, diabetes and hypercholesterolemia were more frequent in the cases than the control group (Table 1). The mean HMWK levels in the control group (117%) and MI case group (117%, mean difference 0%, 95%CI -4 %to 3%) were similar, whereas the levels were slightly increased in the IS case group (121%, mean difference 4%, 95%CI 0% to 8%). Table 2 shows HMWK levels in relation with classical risk factors in the control group. The levels were higher in those suffering from diabetes (mean difference 14%, 95%CI 1% to 27%) and hypercholesterolemia (mean difference 8%, 95%CI -1% to 18%), whereas hypertension, previous OC use and previous smoking habits were not associated with substantial changes in HMWK levels.

The risk of myocardial infarction was not affected by high levels (≥p90) of HWMK (unadjusted OR 1.26, 95%CI 0.75-2.10; adjusted OR 1.05, 95%CI 0.57-1.91) (table 3). There was an association with the risk of ischaemic stroke, which remained after adjustment for all confounders (adjusted OR 1.82, 95%CI 1.00-3.29). Low levels of HMWK were weakly associated both with the risk of myocardial infarction and ischaemic stroke (MI: adjusted OR 1.39, 95%CI 0.74-2.61; IS: 1.49, 95%CI 0.77-2.89). Table 4 shows that there was no clear pattern of increasing risk with increasing levels for myocardial infarction, while the risk of ischaemic stroke was best represented by a U-curve. A post-hoc analysis with 5 categories can be found in table 5.

Interaction analyses with oral contraceptive use in the year prior to the event are shown in table 5. As expected, the risk in the group of women who were only exposed to oral contraceptive use but had normal HWMK levels (i.e. the -/+ group) was about two-threefold increased for both myocardial infarction and ischaemic stroke when compared with women with neither risk factor. The risks associated with high HWMK levels and no OC use were similar to the risks observed in the primary analyses: after adjustment, the

Table 4. HWMK levels categorised in quartiles in relation to the risk of myocardial infarction and ischaemic stroke

	Control		Myocardial infarction						Ischaemic stroke					
	N	%	N	%	OR_1	95%CI	OR_2	95%CI	N	%	OR_1	95%CI	OR_2	95%CI
Q1	154	0.25	53	0.27	1	[ref]	1	[ref]	41	0.25	1	[ref]	1	[ref]
Q2	146	0.24	39	0.20	0.75	(0.46-1.24)	0.77	(0.44-1.37)	26	0.16	0.57	(0.32-1.03)	0.51	(0.27-0.97)
Q3	157	0.25	55	0.28	0.82	(0.51-1.31)	0.97	(0.56-1.67)	39	0.24	0.67	(0.38-1.17)	0.71	(0.38-1.30)
Q4	161	0.26	47	0.24	0.72	(0.44-1.17)	0.75	(0.42-1.33)	57	0.35	1.01	(0.60-1.71)	1.00	(0.56-1.78)

See table 3 for description

Table 3. High and low levels of HWMK and the risk of myocardial infarction and ischaemic stroke

		Control		Myocardial infarction						Ischaemic stroke					
		N	%	N	%	OR_1	(95%CI)	OR_2	(95%CI)	N	%	OR_1	(95%CI)	OR_2	(95%CI)
High	**<p90**	554	0.90	169	0.87	1	[ref]	1	[ref]	137	0.83	1	[ref]	1	[ref]
	≥p90	64	0.10	25	0.13	1.26	(0.75-2.10)	1.05	(0.57-1.91)	29	0.17	1.69	(0.99-2.89)	1.82	(1.00-3.29)
Low	**>p10**	557	0.90	170	0.88	1	[ref]	1	[ref]	143	0.88	1	[ref]	1	[ref]
	≤p10	61	0.10	24	0.12	1.69	(0.98-2.89)	1.39	(0.74-2.61)	20	0.12	1.62	(0.88-2.99)	1.49	(0.77-2.89)

Abbreviations: OR = odds ratio, 95% CI = 95% confidence interval, ref = reference group, p10 = 10^{th} percentile of control group, p90 = 90^{th} percentile of control group. OR_1 = odds ratios adjusted for stratification factors (i.e. age, area of residence and index year). OR_2 = odds ratios additionally adjusted for potential confounders (i.e. hypertension, diabetes and hypercholesterolemia). Percentages might not add due to rounding.

Table 5. Interaction analyses with OC use

			Control		Myocardial infarction						Ischaemic stroke					
	≥p90	**OC**	N	%	N	%	OR_1	95%CI	OR_2	95%CI	N	%	OR_1	95%CI	OR_2	95%CI
High HMWK	-	-	366	0.59	104	0.54	1	[ref]	1	[ref]	61	0.31	1	[ref]	1	[ref]
	-	+	188	0.30	65	0.34	2.06	(1.37-3.09)	1.75	(1.11-2.77)	73	0.38	2.87	(1.80-4.56)	2.87	(1.73-4.78)
	+	-	47	0.07	14	0.07	0.94	(0.49-1.82)	0.85	(0.40-1.81)	18	0.09	1.89	(0.97-3.70)	2.16	(1.01-4.62)
	+	+	17	0.03	11	0.06	4.90	(2.04-12)	2.90	(1.01-8.26)	11	0.06	4.34	(1.71-11)	3.89	(1.47-10)
	≤p10	**OC**														
Low HMWK	-	-	368	0.60	108	0.56	1	[ref]	1	[ref]	72	0.44	1	[ref]	1	[ref]
	-	+	189	0.31	62	0.32	2.03	(1.35-3.06)	1.64	(1.03-2.61)	71	0.44	2.61	(1.66-4.14)	2.46	(1.47-4.07)
	+	-	45	0.07	10	0.05	1.12	(0.53-2.41)	0.85	(0.35-2.08)	8	0.04	1.35	(0.55-3.33)	1.06	(0.39-2.83)
	+	+	16	0.03	14	0.07	6.55	(2.77-16)	4.53	(1.73-12)	13	0.08	6.24	(2.43-16)	6.70	(2.44-18)

Abbreviations: OC = oral contraceptive use. See table 3 for further description.

risk for myocardial infarction was not affected, whereas the risk for ischaemic stroke remained doubled. The risk associated with the combination of OC use and low HMWK was increased 4.5 fold for myocardial infarction and 6.5 fold for ischaemic stroke.

Discussion

This study indicates that extreme levels of HMWK are associated with an increased risk of ischaemic stroke, whereas the risk of myocardial infarction is only mildly increased with low levels of HMWK. Analysis by HMWK levels suggested a U-shaped curve for ischaemic stroke. The increase in risk was pronounced higher in women with oral contraceptive use.

When these results are compared with our previous results on the intrinsic coagulation proteins, some similarities can be seen: high levels of HMWK, which could result in more activation of the intrinsic coagulation system, are related to an increased risk of ischaemic stroke, whereas the risk of myocardial infarction is not affected. However, the U-shaped risk curve observed for HMWK levels and the risk of ischaemic stroke does not readily comply with this notion. Moreover, due to different roles of HWMK we cannot draw strong conclusions on whether this increase in ischaemic stroke only reflects the activation rate of the intrinsic coagulation proteins.

Our study is the first to investigate the role of HWMK levels in plasma in relation to both myocardial infarction and ischaemic stroke. Our results are not in line with previous studies which focused on myocardial infarction.[39,40] However, since the blood samples in these small studies were collected in the acute phase, their results are subject to reverse causation and should therefore not be interpreted causally. We did not identify prior

TABLE 6. HWMK levels categorised in quintiles in relation to the risk of myocardial infarction and ischaemic stroke

	Control		Myocardial infarction						Ischaemic stroke					
	N	%	N	%	OR_1	95%CI	OR_2	95%CI	N	%	OR_1	95%CI	OR_2	95%CI
Q1	123	0.20	43	0.26	1.33	(0.77-2.29)	1.19	(0.63-2.23)	34	0.21	1.53	(0.81-2.90)	1.51	(0.76-3.00)
Q2	119	0.19	37	0.22	1.02	(0.58-1.78)	1.03	(0.54-1.94)	21	0.13	0.88	(0.44-1.75)	0.74	(0.35-1.57)
Q3	119	0.19	36	0.22	1	[ref]	1	[ref]	26	0.16	1	[ref]	1	[ref]
Q4	128	0.21	37	0.22	0.85	(0.49-1.49)	0.88	(0.47-1.66)	30	0.18	0.90	(0.47-1.72)	0.87	(0.43-1.75)
Q5	129	0.21	41	0.25	0.98	(0.57-1.70)	0.92	(0.49-1.74)	52	0.32	1.85	(1.02-3.39)	1.85	(0.96-3.57)

See table 3 for description

studies which focused on the relation of plasma levels of HMWK and ischaemic stroke. However, some studies on tissue kallikrein levels as well as the bradykinin-receptor indicate that bradykinin is involved in stroke aetiology through the exacerbation of stroke related brain oedema.[38,43,44] Although the biological mechanisms that were investigated as well as the outcome in these studies are not directly comparable to our study, they do suggest that HMWK and especially activation of bradykinin plays a role in the pathophysiology of ischaemic stroke. Unfortunately, our current observational epidemiological study cannot discriminate between the possible mechanisms by which HWMK exerts its risk increasing action and further study is needed to elucidate the underlying mechanisms.

Remarkable results come from the interaction analyses. The analysis of high HMWK levels in combination with oral contraceptive use showed that the observed effect on ischaemic stroke was present for both users and non-users. All other analyses (i.e. low HMWK levels and the risk of ischaemic stroke, low HMWK levels and the risk of myocardial infarction, high HMWK levels and the risk of myocardial infarction) suggested an increase in risk only for women who were used oral contraceptives. This difference could explain in part why there is a difference in the risks conferred by high HWMK levels between myocardial infarction (OR 1.05) and ischaemic stroke (OR 1.82). The value of this observation, however, is unclear; although it is unlikely that bias could be the sole explanation for this observation, there is also no strong direct biological evidence why certain HMWK concentrations would only be harmful in combination with oral contraceptive use.

Our case-control study has some limitations. A major problem in case-control studies in particular is the possibility of reverse causation where cause and effect are mistaken for each other. It is unclear whether HMWK increases or decreases after a major event and could therefore be considered an acute phase reactant which could lead to reverse causation. Our blood samples have been drawn a minimal of 23 months after the event, i.e., after the acute phase, which renders reverse causation unlikely. Survival bias may also play a role in case-control studies. In this study design, blood samples could only be drawn after the event. Therefore, patients who died shortly after the initial diagnosis were not included. This selection of survivors could influence our results only if the etiologic role of HMWK is different between fatal and non-fatal disease. We believe that HMWK levels are not likely to have a large effect on case fatality rate. If such an effect exists, we hypothesise that high HMWK levels lead to an increase in case fatality rate, since several studies

indicated that bradykinin is associated with worse stroke outcome.[38,43,44] Therefore, the results as presented are at most an underestimation of the true effect. Another problem could be confounding; a mechanism in which an observed effect is not caused by the exposure of interest but by a third factor related to the exposure of interest. In our study, strong cardiovascular risk factors (i.e. hypertension, diabetes, hypercholesterolemia, age) were not or only slightly associated with HMWK levels. Therefore we added these risk factors as putative confounders in our fully adjusted model of which the results did not differ substantially from the unadjusted model. We cannot rule out that our analyses are still confounded to some extent, but we expect that the observed effects cannot be completely explained by confounding mechanisms.

Conclusion Our study shows that both high and low levels of HMWK are associated with increased risk of ischaemic stroke in young women. The risk of low HWMK is only present within oral contraceptive users, whereas the risk of increased HWMK was also present in women who did not use oral contraceptives. HWMK does not seem to have a major impact on the risk of myocardial infarction. Further research is needed to determine the exact underlying mechanism and the role of oral contraceptive use in the causal relation between HWMK levels and the risk of ischaemic stroke.

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