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Title page

Title

Urinary albumin excretion as a marker of endothelial dysfunction in migraineurs; the HUNT study, Norway

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Migraine; headache; endothelial dysfunction; albuminuria

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Abstract

Objective: To investigate urine albumin leakage as a marker of endothelial dysfunction in migraineurs.

Design: A population-based health study

Participants: 304 subjects with migraine, 1025 subjects with non-migraine headache and 5428 headache free controls.

Outcomes: The association between urine albumin to creatinine ratio (ACR) and headache status was investigated in the Nord-Trøndelag Health Study (HUNT-2). Subjects were selected in two strata, based on either 1) self-reported hypertension/diabetes (morbid sample) or 2) a random sample. Analyses were performed using analysis of covariance.

Results: There was no association between headache status and ACR in the study population (p=0.23, mean ACR for migraine 1.66, 95% CI 1.31-2.01, for non-migraine headache 1.90, 95% CI 1.71-2.09 and for no headache 1.73, 95% CI 1.64-1.81) after relevant adjustments. Similarly, no association between headache status and ACR was seen when the analysis was stratified for morbid and random samples, or for migraine with and without aura.

Conclusions: We found no evidence of increased urine albumin leakage in migraineurs when compared to headache free controls. This could indicate that global endothelial dysfunction is not a prominent feature of migraine.

Article summary

Article focus:

- We aimed to investigate urine albumin to creatinine ratio (ACR) as a marker of endothelial dysfunction in subjects with migraine, non-migraine headache and headache free controls.

Key messages:

- No associations were found between headache status and ACR, implying the absence of large differences in urine albumin leakage between migraineurs or other headache sufferers and headache free controls.
- This may indicate that global endothelial dysfunction is not a prominent feature of migraine.

Strengths and limitations of this study:

- The study is the first to assess albumin leakage as a marker of endothelial dysfunction in migraineurs.
- The study includes a relatively large sample size, objective measurements of albumin leakage and the use of validated headache diagnoses.
- The sample size might be too low to detect ACR differences among migraine subtypes. In particular, there was a limited number of migraineurs with aura in this study.

Introduction

Migraine is a common disabling headache disorder affecting 6-7% of men and 18% of women.[1] It is rated as one of the top 10 most disabling diseases[2, 3] and has been estimated to be the most costly neurological disorder to society.[4] Migraine manifests in headache attacks lasting 4-72 hours characterized by throbbing, pulsating and unilateral headache, often accompanied by nausea and photo- and phonophobia.[5] In up to a third of the patients[6] these attacks may be accompanied by additional neurological aura symptoms separating the disease into migraine with aura (MA) and migraine without aura (MO).[5]

Migraine, in particular MA, has been associated with an increased risk of cardiovascular disease (CVD) including ischemic stroke, ischemic lesions of the brain, myocardial infarction, angina and cardiovascular death.[7-9] While the mechanisms underlying this link remain unknown, multiple explanations have been suggested. Shared environmental- or genetic risk factors could be present, changes in vascular function might contribute to migraine pathophysiology or the opposite; migraine pathophysiology could induce changes in vascular function, or mutual mechanistic pathways may exist.[10]

The vascular endothelium plays an important role in setting vascular tone, regulating vascular permeability, maintaining trombotic balance and regulating fibrinolytic- and inflammatory pathways. A dysfunctional vascular endothelium is often observed in CVD.[11] Interestingly, abnormal control of vascular tone,[12] alterations in arterial structure and function[13] and impaired ability to repair endothelial injury[14] have also been reported in migraine patients. The increase of vasoactive mediators during migraine attacks, including the vasoconstrictive factor ET-1 and the pro-inflammatory factor C-reactive protein (CRP), suggests an

involvement of the endothelium in migraine headache.[15, 16] Endothelial dysfunction could also be implicated in the generation of migraine aura as ET-1 has been shown to produce cortical spreading depression (CSD), the presumed substrate of migraine aura, in rats.[17] Furthermore, migraineurs with aura have an increased risk of thrombotic events. It has been reported raised plasma levels of vWf in migraine patients,[18] a factor that promotes clotting and formation when the endothelium is damaged, further supporting the presence of endothelial dysfunction in migraine. This could explain the link between migraine and CVD and might elucidate pathophysiological mechanisms of migraine.

The presence of subclinical increases in urinary albumin excretion (UAE) is believed to reflect endothelial dysfunction.[19] No previous studies have, however, investigated albumin excretion in migraineurs. The aim of this study was therefore to compare urine albumin to creatinine ratio (ACR), as a measure of UAE, in subjects with migraine, non-migraine headache and headache-free controls.

Methods

Subjects and headache diagnoses

Subjects were selected from the second Nord-Trøndelag Health Study (HUNT 2), a general health survey conducted in 1995-1997. All inhabitants aged ≥20 years residing in the county at the time of screening were invited (n = 92,703) and 65,258 subjects (70.4%) participated [20]. The population in Nord-Trøndelag County is ethnically homogenous with less than 3% non-Caucasians. All participants were mailed a questionnaire and attended a clinical examination, for details see.[20] The questionnaire included questions on previous or current CVD, headache disorders, hypertension, diabetes mellitus and smoking habits. The clinical examination included measurements of height, weight and blood pressure. When attending the health examination, participants received a second questionnaire to complete and return by mail. A total of 9,598 participants were included in a further study in which they donated three urine samples for the determination of ACR. Subjects were included based on 1) the presence of self-reported diabetes mellitus and/or medically treated hypertension (morbid sample) and 2) a 5% randomly selected sample of the total population (random sample). Subjects were originally included in a study designed for investigating UAE in hypertension and diabetes. Participants who answered "yes" to the question "Have you suffered from migraine headache during the last 12 months/last year?" were classified as migraine sufferers, those who answered "yes" to the question "Have you suffered from other types of headache during the last 12 months/last year?" were classified as non-migraine headache sufferers and those who answered "no" comprised the headache-free controls. Classification of headache and migraine disorders in HUNT2 was based on a modified version of the International Classification of Headache Disorders (IHCD), validated by interview diagnoses [21]. Subjects who contributed with information regarding both headache status and ACR were eligible for the present study. Subjects with overt proteinuria, ACR ≥ 25mg/mmol in men and ≥

35mg/mmol in women, one subject with migraine (with aura), 16 subjects with non-migraine headache and 141 subjects without headache, were excluded. In total, 303 subjects with migraine, 1009 subjects with non-migraine headache and 5287 headache free subjects were included in the study, in which the morbid sample constituted 3688 medically treated hypertensive subjects, 647 diabetic subjects and 413 subjects with both medically treated hypertension and diabetes mellitus.

Urine sampling

Participants received a unit with three plastic receptacles for three first morning urine samples, three transport tubes and one envelope for returning the samples by mail to the laboratory. Out of 11661 packs handed out, 9598 (82.3%) participants mailed three samples back to the laboratory.[22] Those who failed to return three samples were excluded. A written instruction describing how to collect urine, information about the screening and a questionnaire concerning urinary tract infection in the previous week, persistent hematuria in the last year and menstruation at the time of collection was included. While the most consistent method for determining UAE is 24-h urine sampling, it has been shown that measuring ACR in one or more morning spot urine samples provides good specificity and sensitivity.[23]

Laboratory procedures

Fresh, non-frozen urine samples were analyzed at the Central Laboratory at Levanger Hospital on a Hitachi 91 Autoanalyzer (Hitachi, Mito, Japan). Urine albumin and creatinine levels were determined using an immunoturbimetric method (antihuman serum albumin;

Dako Norway, Oslo) and Jaffé method, respectively. The ACR, measured in mg/mmol, was used as an expression of UAE.

Consent

Participation in HUNT2 was voluntary and each participant signed a written consent. All surveys and analyses were approved by the Norwegian Data Inspectorate and by the Regional Committee for Medical and Health Research Ethics.

Statistical analysis

For all analyses we used mean ACR from three delivered urine samples, measured in mg/mmol. Comparisons of mean ACR between cases with migraine, non-migraine headache and headache free controls in the morbid, random and combined samples were performed by analysis of covariance (ANCOVA). The confounding effect of potential confounders, i.e. age, sex, body mass index (BMI), smoking, self-reported diabetes and self-reported antihypertensive medication, in the combined sample was calculated by the formula [Mean ACR_{crude}–Mean ACR_{adj}]/Mean ACR_{crude}, where Mean ACR_{crude} was the mean ACR in the crude model and Mean ACR_{adj} was the mean ACR in the model adjusted for the relevant variable. Variables with a confounding effect > 5% in one or more headache group were included in the final model. A *p*-value less than 0.05 was used to indicate statistical significance.

Results

Baseline characteristics for the random and morbid samples are shown in table 1. The morbid sample was characterized by a higher age and BMI and a lower proportion of smokers compared to the random sample. For both samples a higher proportion of women had headache than men, with the highest percentage of women found among migraineurs. Mean age was lower in the headache groups than in controls.

Crude analyses indicated an association between ACR and headache status in the morbid sample (p=0.013, mean ACR for migraine 1.22, for non-migraine headache 1.95 and for no headache 2.03) and the random sample (p=0.011, mean ACR for migraine 0.85, for non-migraine headache 0.96 and for no headache 1.22), table 2. However, when adjusting for age and sex this effect disappeared (Model I). No significant associations were observed between headache status and ACR in the morbid sample (p=0.11, mean ACR for migraine 1.87, for non-migraine headache 2.27 and for no headache 1.96) or the random sample (p=0.55, mean ACR for migraine 1.00, for non-migraine headache 1.07 and for no headache 1.16) when adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication (Model II).

A similar pattern was observed when morbid and random samples were combined; crude data indicated an association between ACR and headache status, but this effect was explained by differences in age and sex, table 3. No significant associations were observed when adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication (p=0.23, mean ACR for migraine 1.66, for non-migraine headache 1.90 and for no headache 1.73). Mean differences for ACR in the combined sample (Model II) were -0.07 (95% CI -0.43-0.30) between migraineurs and headache-free controls and 0.18 (95% CI -0.04-0.39)

between non-migraine headache and headache free controls. We also examined the association between headache status and ACR after stratification of migraine into its two subtype's MA and MO. In the adjusted model, no differences in ACR scores were found between either migraine subtype and controls (p=0.68, mean ACR for MA 1.36, for MO 1.73 and for no headache 1.73), table 4. There was no interaction between headache status and self-reported diabetes, or between headache status and self-reported use of antihypertensive medication on ACR. Since the measurement ACR was positively skewed, we repeated the analyses after square root transformation, which provided similar results. To ease interpretation only non-transformed results are presented.

Discussion

The present study did not find an association between headache status and ACR, indicating the absence of large differences in urine albumin leakage between migraineurs or other headache sufferers and headache free controls. The mechanisms linking migraine and cardiovascular disease are likely complex, but dysregulation of vascular endothelium as expressed by albumin leakage is not supported by the present data to be a major factor.

The study includes a relatively large sample size, objective measurements of UAE, analyses based on fresh urine samples and the use of validated headache diagnoses. Furthermore, the design of the study allowed us to evaluate the association between headache status and UAE in a random sample as well as a morbid sample, which included a high proportion of subjects with hypertension and diabetes type I and II, well known risk factors for increased UAE.[24-26] A limitation of the study is, however, that hypertension- and diabetes information was based on self-report. In addition, the sample size might be too low to detect ACR differences among migraine subtypes. In particular, there was a limited number of migraineurs with aura in this study.

A high level of ACR is a risk marker for cardiovascular morbidity and mortality.[27] Abnormal albumin levels in urine have also been reported in inflammatory, non-cardiovascular conditions such as rheumatoid arthritis, inflammatory bowel syndrome and surgery, which has been hypothesized to be a result of circulating inflammatory molecules damaging parenchymal functions of the kidneys [19]. Interestingly, ACR levels even within the normal range have been associated with an increased rate of kidney disease, cardiovascular disease and mortality.[28-30] As UAE is a biological continuum, we used continuous ACR values in the

present study rather than applying microalbuminuria commonly defined as ACR ≥ 2.5mg/mmol. Mean ACR levels in the morbid group of the present study were twice as high as for the randomly chosen sample, which is explained by the presence of hypertension and diabetes. In the random sample, however, ACR was generally low for all headache and headache-free groups.

The unadjusted analysis indicated an association between ACR and headache status in the morbid-, random and combined samples. However, when adjusting for age and sex the association disappeared, suggesting these variables to be the major contributing factors for differences in ACR in the present study. Given the fact that women have less muscular mass and lower creatinine excretion than men, and that creatinine excretion decreases with age,[22] a confounding effect of age and sex on ACR was not unexpected.

Contrary to our hypothesis we did not find urine albumin leakage to be more prominent among migraineurs than among headache-free subjects. If endothelial dysfunction with albumin leakage was a feature of migraine, we would expect elevated ACR levels among migraineurs, and highest among MA. Although we could not find an association between ACR and migraine status, there was a non-significant tendency in the opposite direction; subjects with MA had the lowest ACR levels. While many previous studies favor the presence of endothelial dysfunction in migraineurs, some studies argue against. In fact, endothelium-dependent vasodilation, nitric oxide levels and stimulated t-PA levels have been found similarly expressed in migraineurs and healthy controls.[31-33] Furthermore, it was recently suggested that extracranial dilatation may not be relevant for migraine pain during attacks.[34]

In summary, in this first study examining albumin leakage in migraineurs, we did not find urine albumin leakage to be more prominent among migraineurs than among headache-free subjects, which could indicate that global endothelial dysfunction is not a prominent feature of migraine.

Contributors:

LMJ contributed to the study design, data analysis, data interpretations and wrote the manuscript. BSW contributed to the data analysis, data interpretations and the drafting of the manuscript. SR and JH conceived the original study, provided laboratory resources and contributed to the manuscript. AHP contributed to the data analysis, data interpretations and the manuscript. JAZ contributed to the study design, data interpretations and the manuscript. All authors approved the final version of the manuscript.

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Competing interests:

None

Ethics approval:

The Norwegian Data Inspectorate and the Regional Committee for Medical and Health Research Ethics approved the study.

Provenance and peer review

Not commissioned; externally peer reviewed

Data sharing statement

No additional data are available.

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Table 1: Clinical profile of the study participants

	No headache	Non-migraine headache	Migraine
Morbid sample (n=4748)			
Women (%)	51.7	60.2	69.8
Mean age (SD)	67.0 (11.2)	58.7 (12.9)	51.5 (12.5)
Antihypertensive use (%)	86.9	84.4	83.0
Diabetes (%)	22.5	22.5	20.9
Daily smokers (%)	19.3	24.6	23.1
Mean Body-mass index (SD)	28.6 (4.5)	29.0 (4.9)	28.7 (4.8)
Random sample (n=1851)			
Women (%)	46.8	63.2	75.0
Mean age (SD)	53.9 (16.5)	43.6 (13.7)	39.6 (11.1)
Antihypertensive use (%)	13.1	7.2	7.0
Diabetes (%)	4.1	1.2	0.0
Daily smokers (%)	27.3	33.3	34.8
Mean Body-mass index (SD)	26.5 (4.0)	26.1 (4.0)	25.8 (3.8)

SD: standard deviation.

Table 2. Albumin/Creatinine ratio by headache status and selection criterium

	Crude		**Model I**[C]		**Model II**[D]	
	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value
Morbid sample		0.013[A]		0.13[A]		0.11[A]
No headache	2.03 (1.92-2.14) 3998		1.96 (1.85-2.07) 3998		1.96 (1.85-2.06) 3978	
Non-migraine headache	1.95 (1.68-2.23) 591	0.61[B]	2.26 (1.97-2.54) 591	0.057[B]	2.27 (1.99-2.55) 585	0.044[B]
Migraine	1.22 (0.68-1.75) 159	0.003[B]	1.85 (1.31-2.40) 159	0.70[B]	1.87 (1.33-2.41) 158	0.75[B]
Random sample		0.011[A]		0.60[A]		0.55[A]
No headache	1.22 (1.11-1.32) 1289		1.16 (1.06-1.27) 1289		1.16 (1.05-1.26) 1285	
Non-migraine headache	0.96 (0.77-1.14) 418	0.017[B]	1.08 (0.89-1.26) 418	0.44[B]	1.07 (0.89-1.26) 417	0.44[B]
Migraine	0.85 (0.53-1.16) 144	0.028[B]	1.02 (0.70-1.34) 144	0.41[B]	1.00 (0.68-1.31) 143	0.35[B]

[A]p-value for overall effect of headache groups.
[B]p-values are for comparison against no headache group within the same selection criterium.
[C]Model I: Adjusted for age and sex.
[D]Model II: Adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication.

Table 3: Albumin/Creatinine ratio by headache status in combined sample

	Crude		**Model I**[C]		**Model II**[D]	
	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value
		<0.001[A]		0.29[A]		0.23[A]
No headache	1.83 (1.75-1.92) 5287		1.73 (1.65-1.81) 5287		1.73 (1.64-1.81) 5263	
Non-migraine headache	1.54 (1.35-1.73) 1009	0.007[B]	1.89 (1.69-2.09) 1009	0.15	1.90 (1.71-2.09) 1002	0.11[B]
Migraine	1.04 (0.69-1.39) 303	<0.001[B]	1.67 (1.31-2.03) 303	0.74	1.66 (1.31-2.01) 301	0.72[B]

[A]p-value for overall effect of headache groups.
[B]p-values are for comparison against no headache group.
[C]Model I: Adjusted for age and sex.
[D]Model II: Adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication.

Table 4: Albumin/Creatinine ratio by migraine subtypes

	Crude		**Model I**[C]		**Model II**[D]	
	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value
		<0.001[A]		0.78[A]		0.68[A]
No headache	1.83 (1.75-1.92) 5287		1.73 (1.65-1.81) 5287		1.73 (1.64-1.81) 5263	
Migraine without aura	1.05 (0.66-1.44) 244	<0.001[B]	1.73 (1.33-2122) 244	0.88[B]	1.73 (1.34-2.13) 242	0.85[B]
Migraine with aura	1.00 (0.21-1.80) 59	0.042[B]	1.43 (0.64-2.22) 59	0.49[B]	1.36 (0.59-2.13) 59	0.39[B]

[A]p-value for overall effect of headache groups.
[B]p-values are for comparison against controls.
[C]Model I: Adjusted for age and sex.
[D]Model II: Adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication.

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Urinary albumin excretion as a marker of endothelial dysfunction in migraine sufferers; the HUNT study, Norway

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Abstract

Objective: To investigate urine albumin leakage as a marker of endothelial dysfunction in migraine subjects.

Design: A population-based health study

Participants: 303 subjects with migraine, 1009 subjects with non-migraine headache and 5287 headache free controls.

Outcomes: The association between urine albumin to creatinine ratio (ACR) and headache status was investigated in the Nord-Trøndelag Health Study (HUNT-2). Subjects were selected in two strata, based on either 1) self-reported hypertension/diabetes (morbid sample) or 2) a random sample. Analyses were performed using analysis of covariance.

Results: There was no association between headache status and ACR in the study population (p=0.23, mean ACR for migraine 1.66, 95% CI 1.31-2.01, for non-migraine headache 1.90, 95% CI 1.71-2.09 and for no headache 1.73, 95% CI 1.64-1.81) after relevant adjustments. Similarly, no association between headache status and ACR was seen when the analysis was stratified for morbid and random samples, or for migraine with and without aura.

Conclusions: We found no evidence of increased urine albumin leakage in migraine sufferers when compared to headache free controls. This could indicate that systemic endothelial dysfunction is not a prominent feature of migraine.

Article summary

Article focus:

- We aimed to investigate urine albumin to creatinine ratio (ACR) as a marker of endothelial dysfunction in subjects with migraine, non-migraine headache and headache free controls.

Key messages:

- No associations were found between headache status and ACR, implying the absence of large differences in urine albumin leakage between migraine subjects or other headache sufferers and headache free controls.
- This may indicate that systemic endothelial dysfunction is not a prominent feature of migraine.

Strengths and limitations of this study:

- The study is the first to assess albumin leakage as a marker of endothelial dysfunction in migraine subjects.
- The study includes a relatively large sample size, objective measurements of albumin leakage and the use of validated headache diagnoses.
- The sample size might be too low to detect ACR differences among migraine subtypes. In particular, there was a limited number of subjects having migraine with aura in this study.

Introduction

Migraine is a common disabling headache disorder affecting 6-7% of men and 18% of women.[1] It is rated as one of the top 10 most disabling diseases[2,3] and has been estimated to be the most costly neurological disorder to society.[4] Migraine manifests in headache attacks lasting 4-72 hours characterized by throbbing, pulsating and unilateral headache, often accompanied by nausea and photo- and phonophobia.[5] In up to a third of the patients[6] these attacks may be accompanied by additional neurological aura symptoms separating the disease into migraine with aura (MA) and migraine without aura (MO).[5]

Migraine, in particular MA, has been associated with an increased risk of cardiovascular disease (CVD) including ischemic stroke, ischemic lesions of the brain, myocardial infarction, angina and cardiovascular death.[7-9] While the mechanisms underlying this link remain unknown, multiple explanations have been suggested. Shared environmental- or genetic risk factors could be present, changes in vascular function might contribute to migraine pathophysiology or the opposite; migraine pathophysiology could induce changes in vascular function, or mutual mechanistic pathways may exist.[10]

The vascular endothelium plays an important role in setting vascular tone, regulating vascular permeability, maintaining trombotic balance and regulating fibrinolytic- and inflammatory pathways. A dysfunctional vascular endothelium is often observed in CVD.[11] Interestingly, abnormal control of systemic vascular tone,[12] alterations in systemic arterial structure and function[13] and impaired ability to repair systemic endothelial injury[14] have also been reported in migraine patients. The increase of systemic vasoactive mediators during migraine attacks, including the vasoconstrictive factor ET-1 and the pro-inflammatory factor C-reactive protein

(CRP), suggests an involvement of the endothelium in migraine headache.[15,16] Endothelial dysfunction could also be implicated in the generation of migraine aura as ET-1 has been shown to produce cortical spreading depression (CSD), the presumed substrate of migraine aura, in rats.[17] Furthermore, migraine patients with aura have an increased risk of thrombotic events. It has been reported raised plasma levels of vWf in migraine patients,[18] a factor that promotes clotting and formation when the endothelium is damaged, further supporting the presence of endothelial dysfunction in migraine. This could explain the link between migraine and CVD and might elucidate pathophysiological mechanisms of migraine.

The presence of subclinical increases in urinary albumin excretion (UAE) has been associated with impaired endothelium-dependent vasodilatation[19] and is believed to reflect endothelial dysfunction.[20,21] No previous studies have, however, investigated albumin excretion in migraine subjects. The aim of this study was therefore to compare urine albumin to creatinine ratio (ACR), as a measure of UAE, in subjects with migraine, non-migraine headache and headache-free controls.

Methods

Subjects and headache diagnoses

Subjects were selected from the second Nord-Trøndelag Health Study (HUNT 2), a general health survey conducted in 1995-1997. All inhabitants aged ≥20 years residing in the county at the time of screening were invited (n = 92,703) and 65,258 subjects (70.4%) participated. [22]. The population in Nord-Trøndelag County is ethnically homogenous with less than 3% non-Caucasians. All participants were mailed a questionnaire and attended a clinical examination, for details see.[22] The questionnaire included questions on previous or current CVD, hypertension, diabetes mellitus and smoking habits as well as 13 headache questions. The clinical examination included measurements of height, weight and blood pressure. When attending the health examination, participants received a second questionnaire to complete and return by mail. The 13 headache questions of the questionnaire were designed mainly to determine whether the individual had headache, the frequency of headache, and to diagnose migraine according to a modified version of the ICHD migraine criteria.[23,24] Subjects who answered "yes" to the question "Have you suffered from headache during the last 12 months?" were classified as headache sufferers. Those who answered "no" comprised the headache free control group. Based on the subsequent 12 headache questions headache sufferers were classified as having migraine if they fulfilled the following three criteria: 1) Headache attacks lasting 4 to 72 hours (less than 4 hours was accepted for those who reported often visual disturbances before headache). 2) Headache with at least one of the following characteristics: pulsating quality, unilateral location, aggravation by physical activity. 3) During headache, at least one of the following: nausea, photophobia or phonophobia. Those within this group who reported often having visual disturbance prior to headache were classified as having MA. Headache sufferers who did not fulfil the criteria for migraine and did not have self-reported migraine were classified as having non-migraine headache.

Headache diagnoses were mutually exclusive. The headache diagnoses were validated by clinical interviews by physicians with long clinical experience in headache disorders. The positive and negative predictive values of the questionnaire-based diagnoses were, respectively 68% and 76% for non-migraine headache, 87% and 75% for migraine, and 100% and 91% for MA. A total of 9,598 participants were included in a further study in which they donated three urine samples for the determination of ACR, originally designed for investigating UAE in hypertension and diabetes. Subjects were included based on 1) the presence of self-reported diabetes mellitus and/or medically treated hypertension (morbid sample) and 2) a 5% randomly selected sample of the total population (random sample). Participants who contributed with information regarding both headache status and ACR were eligible for the present study. Subjects with overt proteinuria, ACR ≥ 25mg/mmol in men and ≥ 35mg/mmol in women, one subject with migraine (with aura), 16 subjects with non-migraine headache and 141 subjects without headache, were excluded. In total, 303 subjects with migraine, 1009 subjects with non-migraine headache and 5287 headache free subjects were included in the study, in which the morbid sample constituted 3688 medically treated hypertensive subjects, 647 diabetic subjects and 413 subjects with both medically treated hypertension and diabetes mellitus.

Urine sampling

Participants received a unit with three plastic receptacles for three first morning urine samples, three transport tubes and one envelope for returning the samples by mail to the laboratory. Out of 11661 packs handed out, 9598 (82.3%) participants mailed three samples back to the laboratory.[25] Those who failed to return three samples were excluded. A written instruction describing how to collect urine, information about the screening and a

questionnaire concerning urinary tract infection in the previous week, persistent hematuria in the last year and menstruation at the time of collection was included. While the most consistent method for determining UAE is 24-h urine sampling, it has been shown that measuring ACR in one or more morning spot urine samples provides good specificity and sensitivity.[26]

Laboratory procedures

Fresh, non-frozen urine samples were analyzed at the Central Laboratory at Levanger Hospital on a Hitachi 91 Autoanalyzer (Hitachi, Mito, Japan). Urine albumin and creatinine levels were determined using an immunoturbimetric method (antihuman serum albumin; Dako Norway, Oslo) and Jaffé method, respectively. The ACR, measured in mg/mmol, was used as an expression of UAE.

Consent

Participation in HUNT2 was voluntary and each participant signed a written consent. All surveys and analyses were approved by the Norwegian Data Inspectorate and by the Regional Committee for Medical and Health Research Ethics.

Statistical analysis

For all analyses we used mean ACR from three delivered urine samples, measured in mg/mmol. Comparisons of mean ACR between cases with migraine, non-migraine headache and headache free controls in the morbid, random and combined samples were performed by

analysis of covariance (ANCOVA). The confounding effect of potential confounders, i.e. age, sex, body mass index (BMI), smoking, self-reported diabetes and self-reported antihypertensive medication, in the combined sample was calculated by the formula [Mean ACR_{crude}–Mean ACR_{adj}]/Mean ACR_{crude}, where Mean ACR_{crude} was the mean ACR in the crude model and Mean ACR_{adj} was the mean ACR in the model adjusted for the relevant variable. Variables with a confounding effect > 5% in one or more headache group were included in the final model. A *p*-value less than 0.05 was used to indicate statistical significance.

Results

Baseline characteristics for the random and morbid samples are shown in table 1. The morbid sample was characterized by a higher age and BMI and a lower proportion of smokers compared to the random sample. For both samples a higher proportion of women had headache than men, with the highest percentage of women found among the migraine subjects. Mean age was lower in the headache groups than in controls.

Crude analyses indicated an association between ACR and headache status in the morbid sample (p=0.013, mean ACR for migraine 1.22, for non-migraine headache 1.95 and for no headache 2.03) and the random sample (p=0.011, mean ACR for migraine 0.85, for non-migraine headache 0.96 and for no headache 1.22), table 2. However, when adjusting for age and sex this effect disappeared (Model I). No significant associations were observed between headache status and ACR in the morbid sample (p=0.11, mean ACR for migraine 1.87, for non-migraine headache 2.27 and for no headache 1.96) or the random sample (p=0.55, mean ACR for migraine 1.00, for non-migraine headache 1.07 and for no headache 1.16) when adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication (Model II).

A similar pattern was observed when morbid and random samples were combined; crude data indicated an association between ACR and headache status, but this effect was explained by differences in age and sex, table 3. No significant associations were observed when adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication (p=0.23, mean ACR for migraine 1.66, for non-migraine headache 1.90 and for no headache 1.73). Mean differences for ACR in the combined sample (Model II) were -0.07 (95% CI -0.43-0.30) between migraine subjects and headache-free controls and 0.18 (95% CI -0.04-

0.39) between non-migraine headache and headache free controls. We also examined the association between headache status and ACR after stratification of migraine into its two subtype's MA and MO. In the adjusted model, no differences in ACR scores were found between either migraine subtype and controls (p=0.68, mean ACR for MA 1.36, for MO 1.73 and for no headache 1.73), table 4. There was no interaction between headache status and self-reported diabetes, or between headache status and self-reported use of antihypertensive medication on ACR. Since the measurement ACR was positively skewed, we repeated the analyses after square root transformation, which provided similar results. To ease interpretation only non-transformed results are presented.

Discussion

The present study did not find an association between headache status and ACR, indicating the absence of large differences in urine albumin leakage between migraine subjects or other headache sufferers and headache free controls. The mechanisms linking migraine and cardiovascular disease are likely complex, but dysregulation of vascular endothelium as expressed by albumin leakage is not supported by the present data to be a major factor.

The study includes a relatively large sample size, objective measurements of UAE, analyses based on fresh urine samples and the use of validated headache diagnoses. Furthermore, the design of the study allowed us to evaluate the association between headache status and UAE in a random sample as well as a morbid sample, which included a high proportion of subjects with hypertension and diabetes type I and II, well known risk factors for increased UAE.[27-29] A limitation of the study is, however, that hypertension- and diabetes information was based on self-report. Data on subjects' migraine status (ictal or interictal phase) during urine sampling was not available and it cannot be excluded that there may be a difference in urine albumin leakage during and outside a migraine attack. In addition, the sample size might be too low to detect ACR differences among migraine subtypes. In particular, there was a limited number of subjects having migraine with aura in this study.

A high level of urine ACR, most likely representing excessive glomerular capillary leakage of albumin,[19] is a risk marker for cardiovascular morbidity and mortality[30] and is believed to reflect endothelial dysfunction.[19,21] Increased UAE has been associated with impaired endothelium-dependent vasodilatation[19], impaired peripheral vasoreactivity to endothelial agonists[31] as well as with circulating biomarkers of endothelial dysfunction.[32] Abnormal ACR

levels in urine have also been reported in inflammatory, non-cardiovascular conditions such as rheumatoid arthritis, inflammatory bowel syndrome and surgery, which has been hypothesized to be a result of circulating inflammatory molecules damaging parenchymal functions of the kidneys.[20] Interestingly, ACR levels even within the normal range have been associated with an increased rate of kidney disease, cardiovascular disease and mortality.[33-35] As UAE is a biological continuum, we used continuous ACR values in the present study rather than applying microalbuminuria commonly defined as ACR ≥ 2.5mg/mmol. Mean ACR levels in the morbid group of the present study were twice as high as for the randomly chosen sample, which is explained by the presence of hypertension and diabetes. In the random sample, however, ACR was generally low for all headache and headache-free groups.

The unadjusted analysis indicated an association between ACR and headache status in the morbid-, random and combined samples. However, when adjusting for age and sex the association disappeared, suggesting these variables to be the major contributing factors for differences in ACR in the present study. Given the fact that women have less muscular mass and lower creatinine excretion than men, and that creatinine excretion decreases with age,[25] a confounding effect of age and sex on ACR was not unexpected.

Contrary to our hypothesis we did not find urine albumin leakage to be more prominent among migraine sufferers than among headache-free subjects. If endothelial dysfunction with albumin leakage was a feature of migraine, we would expect elevated ACR levels among migraine subjects, and highest among MA. Although we could not find an association between ACR and migraine status, there was a non-significant tendency in the opposite direction; subjects with MA had the lowest ACR levels. While many previous studies favor

the presence of endothelial dysfunction in migraine patients, some studies argue against. In fact, systemic endothelium-dependent vasodilation, nitric oxide levels and stimulated t-PA levels have been found similarly expressed in migraine subjects and healthy controls.[36-38] Furthermore, it was recently suggested that extracranial dilatation may not be relevant for migraine pain during attacks.[39]

In summary, in this first study examining albumin leakage in migraine sufferers, we did not find urine albumin leakage to be more prominent among migraine subjects than among headache-free subjects, which could indicate that systemic endothelial dysfunction is not a prominent feature of migraine.

Contributors:

LMJ contributed to the study design, data analysis, data interpretations and wrote the manuscript. BSW contributed to the data analysis, data interpretations and the drafting of the manuscript. SR and JH conceived the original study, provided laboratory resources and contributed to the manuscript. AHP contributed to the data analysis, data interpretations and the manuscript. JAZ contributed to the study design, data interpretations and the manuscript. All authors approved the final version of the manuscript.

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Competing interests:

None

Ethics approval:

The Norwegian Data Inspectorate and the Regional Committee for Medical and Health Research Ethics approved the study.

Provenance and peer review

Not commissioned; externally peer reviewed

Data sharing statement

No additional data are available.

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Table 1: Clinical profile of the study participants

	No headache	Non-migraine headache	Migraine
Morbid sample (n=4748)			
Women (%)	51.7	60.2	69.8
Mean age (SD)	67.0 (11.2)	58.7 (12.9)	51.5 (12.5)
Antihypertensive use (%)	86.9	84.4	83.0
Diabetes (%)	22.5	22.5	20.9
Daily smokers (%)	19.3	24.6	23.1
Mean Body-mass index (SD)	28.6 (4.5)	29.0 (4.9)	28.7 (4.8)
Random sample (n=1851)			
Women (%)	46.8	63.2	75.0
Mean age (SD)	53.9 (16.5)	43.6 (13.7)	39.6 (11.1)
Antihypertensive use (%)	13.1	7.2	7.0
Diabetes (%)	4.1	1.2	0.0
Daily smokers (%)	27.3	33.3	34.8
Mean Body-mass index (SD)	26.5 (4.0)	26.1 (4.0)	25.8 (3.8)

SD: standard deviation.

Table 2. Albumin/Creatinine ratio by headache status and selection criterium

	Crude		**Model I**[C]		**Model II**[D]	
	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value
Morbid sample		0.013[A]		0.13[A]		0.11[A]
No headache	2.03 (1.92-2.14) 3998		1.96 (1.85-2.07) 3998		1.96 (1.85-2.06) 3978	
Non-migraine headache	1.95 (1.68-2.23) 591	0.61[B]	2.26 (1.97-2.54) 591	0.057[B]	2.27 (1.99-2.55) 585	0.044[B]
Migraine	1.22 (0.68-1.75) 159	0.003[B]	1.85 (1.31-2.40) 159	0.70[B]	1.87 (1.33-2.41) 158	0.75[B]
Random sample		0.011[A]		0.60[A]		0.55[A]
No headache	1.22 (1.11-1.32) 1289		1.16 (1.06-1.27) 1289		1.16 (1.05-1.26) 1285	
Non-migraine headache	0.96 (0.77-1.14) 418	0.017[B]	1.08 (0.89-1.26) 418	0.44[B]	1.07 (0.89-1.26) 417	0.44[B]
Migraine	0.85 (0.53-1.16) 144	0.028[B]	1.02 (0.70-1.34) 144	0.41[B]	1.00 (0.68-1.31) 143	0.35[B]

[A]p-value for overall effect of headache groups.
[B]p-values are for comparison against no headache group within the same selection criterium.
[C]Model I: Adjusted for age and sex.
[D]Model II: Adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication.

Table 3: Albumin/Creatinine ratio by headache status in combined sample

	Crude		**Model I**[C]		**Model II**[D]	
	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value
		<0.001[A]		0.29[A]		0.23[A]
No headache	1.83 (1.75-1.92) 5287		1.73 (1.65-1.81) 5287		1.73 (1.64-1.81) 5263	
Non-migraine headache	1.54 (1.35-1.73) 1009	0.007[B]	1.89 (1.69-2.09) 1009	0.15	1.90 (1.71-2.09) 1002	0.11[B]
Migraine	1.04 (0.69-1.39) 303	<0.001[B]	1.67 (1.31-2.03) 303	0.74	1.66 (1.31-2.01) 301	0.72[B]

[A]p-value for overall effect of headache groups.
[B]p-values are for comparison against no headache group.
[C]Model I: Adjusted for age and sex.
[D]Model II: Adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication.

Table 4: Albumin/Creatinine ratio by migraine subtypes

	Crude		**Model I**[C]		**Model II**[D]	
	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value
		<0.001[A]		0.78[A]		0.68[A]
No headache	1.83 (1.75-1.92) 5287		1.73 (1.65-1.81) 5287		1.73 (1.64-1.81) 5263	
Migraine without aura	1.05 (0.66-1.44) 244	<0.001[B]	1.73 (1.33-2122) 244	0.88[B]	1.73 (1.34-2.13) 242	0.85[B]
Migraine with aura	1.00 (0.21-1.80) 59	0.042[B]	1.43 (0.64-2.22) 59	0.49[B]	1.36 (0.59-2.13) 59	0.39[B]

[A]p-value for overall effect of headache groups.
[B]p-values are for comparison against controls.
[C]Model I: Adjusted for age and sex.
[D]Model II: Adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication.

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Title

Urinary albumin excretion as a marker of endothelial dysfunction in migraine sufferers; the HUNT study, Norway

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Key words

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Abstract

Objective: To investigate urine albumin leakage as a marker of endothelial dysfunction in migraine subjects.

Design: A population-based health study

Participants: 303 subjects with migraine, 1009 subjects with non-migraine headache and 5287 headache free controls.

Outcomes: The association between urine albumin to creatinine ratio (ACR) and headache status was investigated in the Nord-Trøndelag Health Study (HUNT-2). Subjects were selected in two strata, based on either 1) self-reported hypertension/diabetes (morbid sample) or 2) a random sample. Analyses were performed using analysis of covariance.

Results: There was no association between headache status and ACR in the study population (p=0.23, mean ACR for migraine 1.66, 95% CI 1.31-2.01, for non-migraine headache 1.90, 95% CI 1.71-2.09 and for no headache 1.73, 95% CI 1.64-1.81) after relevant adjustments. Similarly, no association between headache status and ACR was seen when the analysis was stratified for morbid and random samples, or for migraine with and without aura.

Conclusions: We found no evidence of increased urine albumin leakage in migraine sufferers when compared to headache free controls. This could indicate that systemic endothelial dysfunction is not a prominent feature of migraine.

Article summary

Article focus:

- We aimed to investigate urine albumin to creatinine ratio (ACR) as a marker of endothelial dysfunction in subjects with migraine, non-migraine headache and headache free controls.

Key messages:

- No associations were found between headache status and ACR, implying the absence of large differences in urine albumin leakage between migraine subjects or other headache sufferers and headache free controls.
- This may indicate that systemic endothelial dysfunction is not a prominent feature of migraine.

Strengths and limitations of this study:

- The study is the first to assess albumin leakage as a marker of endothelial dysfunction in migraine subjects.
- The study includes a relatively large sample size, objective measurements of albumin leakage and the use of validated headache diagnoses.
- The sample size might be too low to detect ACR differences among migraine subtypes. In particular, there was a limited number of subjects having migraine with aura in this study.

Introduction

Migraine is a common disabling headache disorder affecting 6-7% of men and 18% of women.[1] It is rated as one of the top 10 most disabling diseases[2,3] and has been estimated to be the most costly neurological disorder to society.[4] Migraine manifests in headache attacks lasting 4-72 hours characterized by throbbing, pulsating and unilateral headache, often accompanied by nausea and photo- and phonophobia.[5] In up to a third of the patients[6] these attacks may be accompanied by additional neurological aura symptoms separating the disease into migraine with aura (MA) and migraine without aura (MO).[5]

Migraine, in particular MA, has been associated with an increased risk of cardiovascular disease (CVD) including ischemic stroke, ischemic lesions of the brain, myocardial infarction, angina and cardiovascular death.[7-9] While the mechanisms underlying this link remain unknown, multiple explanations have been suggested. Shared environmental- or genetic risk factors could be present, changes in vascular function might contribute to migraine pathophysiology or the opposite; migraine pathophysiology could induce changes in vascular function, or mutual mechanistic pathways may exist.[10]

The vascular endothelium plays an important role in setting vascular tone, regulating vascular permeability, maintaining trombotic balance and regulating fibrinolytic- and inflammatory pathways. A dysfunctional vascular endothelium is often observed in CVD.[11] Interestingly, abnormal control of systemic vascular tone,[12] alterations in systemic arterial structure and function[13] and impaired ability to repair systemic endothelial injury[14] have also been reported in migraine patients. The increase of systemic vasoactive mediators during migraine attacks, including the vasoconstrictive factor ET-1 and the pro-inflammatory factor C-reactive protein

(CRP), suggests an involvement of the endothelium in migraine headache.[15,16] Endothelial dysfunction could also be implicated in the generation of migraine aura as ET-1 has been shown to produce cortical spreading depression (CSD), the presumed substrate of migraine aura, in rats.[17] Furthermore, migraine patients with aura have an increased risk of thrombotic events. It has been reported raised plasma levels of vWf in migraine patients,[18] a factor that promotes clotting and formation when the endothelium is damaged, further supporting the presence of endothelial dysfunction in migraine. This could explain the link between migraine and CVD and might elucidate pathophysiological mechanisms of migraine.

The presence of subclinical increases in urinary albumin excretion (UAE) has been associated with impaired endothelium-dependent vasodilatation[19] and is believed to reflect endothelial dysfunction.[20,21] No previous studies have, however, investigated albumin excretion in migraine subjects. The aim of this study was therefore to compare urine albumin to creatinine ratio (ACR), as a measure of UAE, in subjects with migraine, non-migraine headache and headache-free controls.

Methods

Subjects and headache diagnoses

Subjects were selected from the second Nord-Trøndelag Health Study (HUNT 2), a general health survey conducted in 1995-1997. All inhabitants aged ≥20 years residing in the county at the time of screening were invited (n = 92,703) and 65,258 subjects (70.4%) participated. [22]. The population in Nord-Trøndelag County is ethnically homogenous with less than 3% non-Caucasians. All participants were mailed a questionnaire and attended a clinical examination, for details see.[22] The questionnaire included questions on previous or current CVD, hypertension, diabetes mellitus and smoking habits as well as 13 headache questions. The clinical examination included measurements of height, weight and blood pressure. When attending the health examination, participants received a second questionnaire to complete and return by mail. The 13 headache questions of the questionnaire were designed mainly to determine whether the individual had headache, the frequency of headache, and to diagnose migraine according to a modified version of the ICHD migraine criteria.[23,24] Subjects who answered "yes" to the question "Have you suffered from headache during the last 12 months?" were classified as headache sufferers. Those who answered "no" comprised the headache free control group. Based on the subsequent 12 headache questions headache sufferers were classified as having migraine if they fulfilled the following three criteria: 1) Headache attacks lasting 4 to 72 hours (less than 4 hours was accepted for those who reported often visual disturbances before headache). 2) Headache with at least one of the following characteristics: pulsating quality, unilateral location, aggravation by physical activity. 3) During headache, at least one of the following: nausea, photophobia or phonophobia. Those within this group who reported often having visual disturbance prior to headache were classified as having MA. Headache sufferers who did not fulfil the criteria for migraine and did not have self-reported migraine were classified as having non-migraine headache.

Headache diagnoses were mutually exclusive. The headache diagnoses were validated by clinical interviews by physicians with long clinical experience in headache disorders. The positive and negative predictive values of the questionnaire-based diagnoses were, respectively 68% and 76% for non-migraine headache, 87% and 75% for migraine, and 100% and 91% for MA. A total of 9,598 participants were included in a further study in which they donated three urine samples for the determination of ACR, originally designed for investigating UAE in hypertension and diabetes. Subjects were included based on 1) the presence of self-reported diabetes mellitus and/or medically treated hypertension (morbid sample) and 2) a 5% randomly selected sample of the total population (random sample). Participants who contributed with information regarding both headache status and ACR were eligible for the present study. Subjects with overt proteinuria, ACR ≥ 25mg/mmol in men and ≥ 35mg/mmol in women, one subject with migraine (with aura), 16 subjects with non-migraine headache and 141 subjects without headache, were excluded. In total, 303 subjects with migraine, 1009 subjects with non-migraine headache and 5287 headache free subjects were included in the study, in which the morbid sample constituted 3688 medically treated hypertensive subjects, 647 diabetic subjects and 413 subjects with both medically treated hypertension and diabetes mellitus.

Urine sampling

Participants received a unit with three plastic receptacles for three first morning urine samples, three transport tubes and one envelope for returning the samples by mail to the laboratory. Out of 11661 packs handed out, 9598 (82.3%) participants mailed three samples back to the laboratory.[25] Those who failed to return three samples were excluded. A written instruction describing how to collect urine, information about the screening and a

questionnaire concerning urinary tract infection in the previous week, persistent hematuria in the last year and menstruation at the time of collection was included. While the most consistent method for determining UAE is 24-h urine sampling, it has been shown that measuring ACR in one or more morning spot urine samples provides good specificity and sensitivity.[26]

Laboratory procedures

Fresh, non-frozen urine samples were analyzed at the Central Laboratory at Levanger Hospital on a Hitachi 91 Autoanalyzer (Hitachi, Mito, Japan). Urine albumin and creatinine levels were determined using an immunoturbimetric method (antihuman serum albumin; Dako Norway, Oslo) and Jaffé method, respectively. The ACR, measured in mg/mmol, was used as an expression of UAE.

Consent

Participation in HUNT2 was voluntary and each participant signed a written consent. All surveys and analyses were approved by the Norwegian Data Inspectorate and by the Regional Committee for Medical and Health Research Ethics.

Statistical analysis

For all analyses we used mean ACR from three delivered urine samples, measured in mg/mmol. Comparisons of mean ACR between cases with migraine, non-migraine headache and headache free controls in the morbid, random and combined samples were performed by

analysis of covariance (ANCOVA). The confounding effect of potential confounders, i.e. age, sex, body mass index (BMI), smoking, self-reported diabetes and self-reported antihypertensive medication, in the combined sample was calculated by the formula [Mean ACR_{crude}–Mean ACR_{adj}]/Mean ACR_{crude}, where Mean ACR_{crude} was the mean ACR in the crude model and Mean ACR_{adj} was the mean ACR in the model adjusted for the relevant variable. Variables with a confounding effect > 5% in one or more headache group were included in the final model. A *p*-value less than 0.05 was used to indicate statistical significance.

Results

Baseline characteristics for the random and morbid samples are shown in table 1. The morbid sample was characterized by a higher age and BMI and a lower proportion of smokers compared to the random sample. For both samples a higher proportion of women had headache than men, with the highest percentage of women found among the migraine subjects. Mean age was lower in the headache groups than in controls.

Crude analyses indicated an association between ACR and headache status in the morbid sample (p=0.013, mean ACR for migraine 1.22, for non-migraine headache 1.95 and for no headache 2.03) and the random sample (p=0.011, mean ACR for migraine 0.85, for non-migraine headache 0.96 and for no headache 1.22), table 2. However, when adjusting for age and sex this effect disappeared (Model I). No significant associations were observed between headache status and ACR in the morbid sample (p=0.11, mean ACR for migraine 1.87, for non-migraine headache 2.27 and for no headache 1.96) or the random sample (p=0.55, mean ACR for migraine 1.00, for non-migraine headache 1.07 and for no headache 1.16) when adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication (Model II).

A similar pattern was observed when morbid and random samples were combined; crude data indicated an association between ACR and headache status, but this effect was explained by differences in age and sex, table 3. No significant associations were observed when adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication (p=0.23, mean ACR for migraine 1.66, for non-migraine headache 1.90 and for no headache 1.73). Mean differences for ACR in the combined sample (Model II) were -0.07 (95% CI -0.43-0.30) between migraine subjects and headache-free controls and 0.18 (95% CI -0.04-

0.39) between non-migraine headache and headache free controls. We also examined the association between headache status and ACR after stratification of migraine into its two subtype's MA and MO. In the adjusted model, no differences in ACR scores were found between either migraine subtype and controls (p=0.68, mean ACR for MA 1.36, for MO 1.73 and for no headache 1.73), table 4. There was no interaction between headache status and self-reported diabetes, or between headache status and self-reported use of antihypertensive medication on ACR. Since the measurement ACR was positively skewed, we repeated the analyses after square root transformation, which provided similar results. To ease interpretation only non-transformed results are presented.

Discussion

The present study did not find an association between headache status and ACR, indicating the absence of large differences in urine albumin leakage between migraine subjects or other headache sufferers and headache free controls. The mechanisms linking migraine and cardiovascular disease are likely complex, but dysregulation of vascular endothelium as expressed by albumin leakage is not supported by the present data to be a major factor.

The study includes a relatively large sample size, objective measurements of UAE, analyses based on fresh urine samples and the use of validated headache diagnoses. Furthermore, the design of the study allowed us to evaluate the association between headache status and UAE in a random sample as well as a morbid sample, which included a high proportion of subjects with hypertension and diabetes type I and II, well known risk factors for increased UAE.[27-29] A limitation of the study is, however, that hypertension- and diabetes information was based on self-report. Data on subjects' migraine status (ictal or interictal phase) during urine sampling was not available and it cannot be excluded that there may be a difference in urine albumin leakage during and outside a migraine attack. In addition, the sample size might be too low to detect ACR differences among migraine subtypes. In particular, there was a limited number of subjects having migraine with aura in this study.

A high level of urine ACR, most likely representing excessive glomerular capillary leakage of albumin,[19] is a risk marker for cardiovascular morbidity and mortality[30] and is believed to reflect endothelial dysfunction.[19,21] Increased UAE has been associated with impaired endothelium-dependent vasodilatation[19], impaired peripheral vasoreactivity to endothelial agonists[31] as well as with circulating biomarkers of endothelial dysfunction.[32] Abnormal ACR

levels in urine have also been reported in inflammatory, non-cardiovascular conditions such as rheumatoid arthritis, inflammatory bowel syndrome and surgery, which has been hypothesized to be a result of circulating inflammatory molecules damaging parenchymal functions of the kidneys.[20] Interestingly, ACR levels even within the normal range have been associated with an increased rate of kidney disease, cardiovascular disease and mortality.[33-35] As UAE is a biological continuum, we used continuous ACR values in the present study rather than applying microalbuminuria commonly defined as ACR ≥ 2.5mg/mmol. Mean ACR levels in the morbid group of the present study were twice as high as for the randomly chosen sample, which is explained by the presence of hypertension and diabetes. In the random sample, however, ACR was generally low for all headache and headache-free groups.

The unadjusted analysis indicated an association between ACR and headache status in the morbid-, random and combined samples. However, when adjusting for age and sex the association disappeared, suggesting these variables to be the major contributing factors for differences in ACR in the present study. Given the fact that women have less muscular mass and lower creatinine excretion than men, and that creatinine excretion decreases with age,[25] a confounding effect of age and sex on ACR was not unexpected.

Contrary to our hypothesis we did not find urine albumin leakage to be more prominent among migraine sufferers than among headache-free subjects. If endothelial dysfunction with albumin leakage was a feature of migraine, we would expect elevated ACR levels among migraine subjects, and highest among MA. Although we could not find an association between ACR and migraine status, there was a non-significant tendency in the opposite direction; subjects with MA had the lowest ACR levels. While many previous studies favor

the presence of endothelial dysfunction in migraine patients, some studies argue against. In fact, systemic endothelium-dependent vasodilation, nitric oxide levels and stimulated t-PA levels have been found similarly expressed in migraine subjects and healthy controls.[36-38] Furthermore, it was recently suggested that extracranial dilatation may not be relevant for migraine pain during attacks.[39]

In summary, in this first study examining albumin leakage in migraine sufferers, we did not find urine albumin leakage to be more prominent among migraine subjects than among headache-free subjects, which could indicate that systemic endothelial dysfunction is not a prominent feature of migraine.

Contributors:

LMJ contributed to the study design, data analysis, data interpretations and wrote the manuscript. BSW contributed to the data analysis, data interpretations and the drafting of the manuscript. SR and JH conceived the original study, provided laboratory resources and contributed to the manuscript. AHP contributed to the data analysis, data interpretations and the manuscript. JAZ contributed to the study design, data interpretations and the manuscript. All authors approved the final version of the manuscript.

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Competing interests:

None

Ethics approval:

The Norwegian Data Inspectorate and the Regional Committee for Medical and Health Research Ethics approved the study.

Provenance and peer review

Not commissioned; externally peer reviewed

Data sharing statement

No additional data are available.

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Table 1: Clinical profile of the study participants

	No headache	Non-migraine headache	Migraine
Morbid sample (n=4748)			
Women (%)	51.7	60.2	69.8
Mean age (SD)	67.0 (11.2)	58.7 (12.9)	51.5 (12.5)
Antihypertensive use (%)	86.9	84.4	83.0
Diabetes (%)	22.5	22.5	20.9
Daily smokers (%)	19.3	24.6	23.1
Mean Body-mass index (SD)	28.6 (4.5)	29.0 (4.9)	28.7 (4.8)
Random sample (n=1851)			
Women (%)	46.8	63.2	75.0
Mean age (SD)	53.9 (16.5)	43.6 (13.7)	39.6 (11.1)
Antihypertensive use (%)	13.1	7.2	7.0
Diabetes (%)	4.1	1.2	0.0
Daily smokers (%)	27.3	33.3	34.8
Mean Body-mass index (SD)	26.5 (4.0)	26.1 (4.0)	25.8 (3.8)

SD: standard deviation.

Table 2. Albumin/Creatinine ratio by headache status and selection criterium

	Crude		**Model I**[C]		**Model II**[D]	
	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value
Morbid sample		0.013[A]		0.13[A]		0.11[A]
No headache	2.03 (1.92-2.14) 3998		1.96 (1.85-2.07) 3998		1.96 (1.85-2.06) 3978	
Non-migraine headache	1.95 (1.68-2.23) 591	0.61[B]	2.26 (1.97-2.54) 591	0.057[B]	2.27 (1.99-2.55) 585	0.044[B]
Migraine	1.22 (0.68-1.75) 159	0.003[B]	1.85 (1.31-2.40) 159	0.70[B]	1.87 (1.33-2.41) 158	0.75[B]
Random sample		0.011[A]		0.60[A]		0.55[A]
No headache	1.22 (1.11-1.32) 1289		1.16 (1.06-1.27) 1289		1.16 (1.05-1.26) 1285	
Non-migraine headache	0.96 (0.77-1.14) 418	0.017[B]	1.08 (0.89-1.26) 418	0.44[B]	1.07 (0.89-1.26) 417	0.44[B]
Migraine	0.85 (0.53-1.16) 144	0.028[B]	1.02 (0.70-1.34) 144	0.41[B]	1.00 (0.68-1.31) 143	0.35[B]

[A]p-value for overall effect of headache groups.
[B]p-values are for comparison against no headache group within the same selection criterium.
[C]Model I: Adjusted for age and sex.
[D]Model II: Adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication.

Table 3: Albumin/Creatinine ratio by headache status in combined sample

	Crude		**Model I**[C]		**Model II**[D]	
	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value
		<0.001[A]		0.29[A]		0.23[A]
No headache	1.83 (1.75-1.92) 5287		1.73 (1.65-1.81) 5287		1.73 (1.64-1.81) 5263	
Non-migraine headache	1.54 (1.35-1.73) 1009	0.007[B]	1.89 (1.69-2.09) 1009	0.15	1.90 (1.71-2.09) 1002	0.11[B]
Migraine	1.04 (0.69-1.39) 303	<0.001[B]	1.67 (1.31-2.03) 303	0.74	1.66 (1.31-2.01) 301	0.72[B]

[A]p-value for overall effect of headache groups.
[B]p-values are for comparison against no headache group.
[C]Model I: Adjusted for age and sex.
[D]Model II: Adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication.

Table 4: Albumin/Creatinine ratio by migraine subtypes

	Crude		**Model I**[C]		**Model II**[D]	
	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value	mean ACR (95% CI) N	p-value
		<0.001[A]		0.78[A]		0.68[A]
No headache	1.83 (1.75-1.92) 5287		1.73 (1.65-1.81) 5287		1.73 (1.64-1.81) 5263	
Migraine without aura	1.05 (0.66-1.44) 244	<0.001[B]	1.73 (1.33-2122) 244	0.88[B]	1.73 (1.34-2.13) 242	0.85[B]
Migraine with aura	1.00 (0.21-1.80) 59	0.042[B]	1.43 (0.64-2.22) 59	0.49[B]	1.36 (0.59-2.13) 59	0.39[B]

[A]p-value for overall effect of headache groups.
[B]p-values are for comparison against controls.
[C]Model I: Adjusted for age and sex.
[D]Model II: Adjusted for age, sex, self-reported diabetes and self-reported use of antihypertensive medication.