

CEREBROVASCULAR REACTIVITY IN PATIENTS WITH CHRONIC RENAL FAILURE ANAEMIA

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REZUMAT

Introducere: Obiectivul lucrării a fost evaluarea reactivității cerebrovasculare (RCV) prin transcranial Doppler (TCD) și testul breath-holding (TBH) la pacienții (p) cu anemie și insuficiență renală cronică (IRC); corelarea răspunsului cerebrovascular la hipercapnie cu severitatea anemiei. **Material:** Studiul a fost efectuat pe un grup de 23 pacienți cu IRC [(9p- glomerulonefrită cronică; 11p- pielonefrită cronică; 3p- LES), divizat în 2 subgrupuri: A- anemie moderată (Hb= 8-11 g%)- 12p; B- anemie severă (Hb< 8g%)- 11p] și un grup – control de 18 subiecți normali (CN), similar ca sex și vârstă. Criteriile de excludere au fost diabetul zaharat și istoric și/ sau semne actuale de boală cerebrovasculară. RCV a fost evaluată prin TCD, utilizând un velocimetru Doppler (Explorer CVC- DMS- Montpellier, Franta) cu analiză spectrală fast Fourier transformation (sonda 4 MHz CW). TBH a constat din hipercapnie spontană indusă prin apnee timp de 20 secunde. RCV a fost apreciată în relație cu creșterea vitezei medii de flux (VMF) în artera cerebrală medie dreaptă și stângă în timpul hipercapniei, comparativ cu valoarea vitezei medii bazale (VMB); semnificația statistică a fost considerată la $P<0.05$. **Rezultate:** În cursul TBH creșterea VMF a fost semnificativă la CN ($P<0.001$), moderată la subgrupul A ($P<0.001$) și foarte redusă la subgrupul B ($P<0.01$), comparativ cu grupul CN; analiza univariată ANOVA între CN și subgrupurile A și B a arătat o diferență semnificativă a RCV între cele 3 loturi ($P<0.01$). Analiza regresivă liniară (Pearson) a relevat o corelație directă între creșterea VMF și nivelul Hb ($r=0.94$; $P<0.001$) și al Hct ($r=0.85$; $P<0.0001$). **Concluzii:** RCV este perturbată la pacienții cu anemie și IRC. Capacitatea vasodilatatorie cerebrală este scăzută în cursul hipercapniei induse prin TBH. Aceste modificări hemodinamice cerebrale se corelează semnificativ cu severitatea anemiei. Hipoxia cronică datorată anemiei din IRC poate fi responsabilă de perturbarea RCV.

Cuvinte cheie: anemie, testul breath-holding, insuficiența renală cronică, reactivitate cerebro-vasculară, ecografie transcranială Doppler

ABSTRACT

Introduction: The cerebrovascular reactivity (CVR) is a haemodynamic parameter which represents the normal increase of the cerebral artery blood flow in response to a vasodilator stimulus, such as hypercapnia. The aim of the study was to assess the CVR by transcranial Doppler (TCD) ultrasound and the breath-holding test (BHT) in patients with chronic renal failure (CRF) anaemia; to correlate the cerebrovascular response to hypercapnia with the severity of anaemia. **Material and methods:** The study was conducted on a group of 23 patients (p) with pre-dialysis- CRF [divided in 2 subgroups: A- mild anaemia (Hb=8-11g/dl)- 12p; B-severe anaemia (Hb<8g/dl)-11p] and a group of 18 gender- and age- matched normal controls (NC). Exclusion criteria were diabetes mellitus and present or past history of cerebrovascular disease. The BHT consisted of spontaneous hypercapnia induced by holding breath for 20 seconds. The CVR was estimated in relationship with the increase in the mean flow velocity (MFV) in the right and the left middle cerebral arteries during hypercapnia as compared to the basal velocity. **Results:** During the BHT the increase in the MFV was significant in NC ($P<0.001$), mild in subgroup A ($P<0.001$) and very slight in subgroup B ($P<0.01$); one-way ANOVA comparison between NC, subgroups A and B showed a significance of $P<0.01$. Linear correlation analysis (Pearson) revealed a direct correlation between the increase in the MFV and the values of Hb ($r=0.94$; $P<0.001$) and of Hct ($r=0.85$; $P<0.0001$). **Conclusion:** The CVR is impaired in patients with CRF and anaemia. These cerebral haemodynamic changes correlate significantly with the severity of anaemia.

Key Words: anaemia, breath-holding test, chronic renal failure, cerebrovascular reactivity, transcranial Doppler ultrasonography.

INTRODUCTION

Cardiovascular complications are the main cause of mortality in patients with end-stage renal disease

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(ESRD), in whom 40% of deaths may be attributed to cardiac disease. The most frequent cardiovascular alterations in patients with ESRD consist of left ventricular hypertrophy, non-atherosclerotic alterations of large conduit arteries, and occlusive atherosclerotic lesions of medium- sized and small conduit arteries.¹

Apart from haemodynamic factors, anaemia is an additional mechanism involved in the development of the cardiovascular structural and functional alterations described in ESRD patients.²

In the normal brain, the constancy of cerebral blood flow and volume relies on the intrinsic ability

of the cerebral arteries to alter their caliber in response to variations in blood pressure (autoregulation) and changes in regional metabolic demands.³

Under hypoxic circumstances induced by anaemia within the brain tissue, which is the case with ESRD patients, cerebral vessels undergo structural changes, with subsequent impairment in the haemodynamic autoregulation processes.⁴

The cerebrovascular reactivity (CVR) is a haemodynamic parameter which represents normal cerebral artery blood flow velocity increase in response to a vasodilator stimulus, such as hypercapnia. A decreased CVR is indicative of preexisting vasodilation, which reflects a reduced reserve capacity of cerebral autoregulation. The CVR provides information with regard to the intracerebral arterioles which may be already maximally dilated, thus being unable to react to drops in blood pressure or to vasodilatory stimuli with further dilation.⁵

Cerebral haemodynamics and the CVR may be assessed by measuring the changes in cerebral blood flow through several methods, such as: positron emission tomography (PET),⁶ single-photon emission tomography,⁷ xenon computed tomography,⁸ functional magnetic resonance imaging,³ and transcranial Doppler ultrasonography (TCD).⁵

In order to evaluate the CVR by means of these techniques, various vasodilator stimuli are utilized: the increase in the arterial PCO₂ (CO₂ test- by inhaling 5% CO₂ in 95% O₂) or the intravenous administration of acetazolamide (Diamox test).⁵

TCD ultrasonography is a noninvasive and nonradioactive method that enables measurement of blood flow velocities in the main intracranial arteries and flow velocity changes after a vasodilator stimulus, namely hypercapnia. The CVR may be easily assessed through TCD by evaluating the increase in the mean flow velocity in the cerebral arteries as a result to their dilation in response to hypercapnia, which may be induced by the breath-holding test (BHT),⁹⁻¹³ the CO₂ test,⁵ and i.v. acetazolamide.¹⁴

The cerebral vasodilating capacity is impaired in patients with chronic renal failure (CRF) and secondary anaemia. According to the studies carried out in CRF patients with anaemia, the CVR is decreased due to chronic hypoxia and the response to hypercapnia is attenuated, a fact demonstrated through O-15 H₂O PET by Kuwabara et al in predialysis as well as in haemodialysis patients⁴ and by Szprynger et al by TCD ultrasonography in children with CRF on conservative treatment, on CAPD and on haemodialysis (HD).¹⁵

The aim of the current study was to assess the CVR by TCD ultrasonography and the BHT in patients

with pre-dialysis CRF and anaemia. Furthermore, the cerebrovascular response to hypercapnia was evaluated in relationship with the severity of anaemia.

SUBJECT AND METHODS

Patients and controls

The study was conducted on a group of 23 patients with CRF in the pre-dialysis stage and renal anaemia (9 patients- chronic glomerulonephritis, 11 patients- chronic pyelonephritis, 3 patients- SLE). This group was divided in two subgroups, A and B, according to the degree of anaemia, as follows: subgroup A mild anaemia (haemoglobin- Hb= 8-11 g/dl)- 12 patients (females- 58%; males 42%; mean age- 47.61 ± 14.53 years); subgroup B- severe anaemia (Hb< 8 g/dl)- 11 patients (females- 64%, males- 36%, mean age- 49.48 ± 18.31 years). A group of 18 age- and gender-matched normal controls (group C) was utilized in order to compare data obtained in the study groups. Exclusion criteria amongst CRF patients were diabetes mellitus and present or past history and symptoms of cerebrovascular disease.

Transcranial Doppler ultrasonography

TCD ultrasonography was performed with a Doppler velocimeter (Explorer CVC-DMS-Montpellier, France) with fast-Fourier transformation spectral analysis, utilizing a 2 MHz- PW probe, through the transtemporal window, at a depth of 50 mm. Both middle cerebral arteries, right and left, (RMCA, LMCA) were examined separately by evaluating the mean flow velocity (MFV), systolic flow velocity (SFV) and diastolic flow velocity (DFV) at rest (normal respiration). The CVR was assessed during the BHT, such as follows: the patient breaths normally by inhaling the air from the examination room, holding breath thereafter for 20 seconds by the end of a normal inspiration. The MFV, SFV and DFV were monitored for each patient and normal control in the RMCA and LMCA at rest (normal respiration- normocapnia), during the manoeuvre of breath holding, and by the end of the BHT, when the flow velocities reached their maximal values (hypercapnia).

The CVR was estimated in relationship with the increase in the MFV in the RMCA and LMCA during hypercapnia as compared to the basal velocity in cm/sec and in percent (%) increase: normal CVR-% increase >15%; diminished CVR-% increase= 5-10%; exhausted CVR-% increase <5% (from ref. 5, modified).

The CVR values were correlated with the severity of anaemia (Hb- g/dl; haematocrit- Hct %).

Statistical analysis

Data was expressed as means ± SD (SBP, DBP,

Hb, MFV at rest, MFV- BHT, CVR). Statistical analysis was performed with a computerized program (EPI INFO 6, INSTAT). An unpaired Student's t-test was utilized in order to compare two subject groups, and ANOVA was used to assess the significance of difference among three subject groups. Correlation analysis (Pearson's test) was used to assess the significance of the relationship between CVR- Hb and CVR- Hct (CVR estimated as % increase in the MFV). Significance was considered if $P < 0.05$.

RESULTS

Characteristics of the subject groups

Table 1 shows the characteristics of the three subject groups. There were no significant age differences between the three groups. The comparison between groups A-C and B-C concerning Hb showed a significant difference ($P < 0.01$ and $P < 0.001$, respectively). As for Hct, there was a significant difference between groups A and C ($P < 0.01$), and groups B and C ($P < 0.001$). Furthermore, a significant difference was observed for SBP in groups A and C ($P < 0.05$) and groups B and C ($P < 0.05$), as well as for DBP in groups A and C ($P < 0.05$) and in groups B and C ($P < 0.05$).

Assessment of the cerebrovascular reactivity

The increase in the MFV in both middle cerebral arteries was normal in normal subjects, mild in patients

with mild anaemia, and very slight in patients with severe anaemia. There were no differences between RMCA and LMCA.

The percent increase in the MFV during the BHT in patients with mild anaemia was in the range of 5-10% in 2 patients, 11-15% in 6 patients, and 16-20% in 4 patients, respectively. No differences were recorded between the RMCA and LMCA.

The percent increase in the MFV during the BHT in patients with severe anaemia was in the range of <5% in 2 patients, 5-10% in 6 patients, and 11-15% in 3 patients, respectively. The results did not differ between the RMCA and LMCA.

The CVR in normal subjects was remarkably increased, thus underlining the normal response to hypercapnia.

By contrast, in patients with mild anaemia, the CVR was normal in only 33% of patients and diminished in 67%, while in patients with severe anaemia, the CVR was diminished in 82% and exhausted in 18%.

Table 2 reveals the differences in the CVR between groups A, B and C. The comparison of the CVR assessed by the increase in the MFV (cm/sec) showed significant differences in groups A-B ($P < 0.05$), A-C ($P < 0.001$), B-C ($P < 0.001$), and A-B-C ($P < 0.01$). The differences in percent increase in the MFV during the BHT were also very significant in groups A-B ($P < 0.01$), A-C ($P < 0.001$), B-C ($P < 0.001$), and A-B-C ($P < 0.01$).

Table 1. Characteristics of groups A, B, and C

| GROUP OF STUDY | Nr. patients | Mean age (years) | Hb (g/dl) | Hct (%) | SBP (mmHg) | DBP (mmHg) | Serum albumin (g%) | Fibrinogen (mg%) | CRP (mg%) |
|----------------|--------------|------------------|---------------|----------------|----------------|---------------|--------------------|------------------|-------------|
| A | 12 | 47.61 ± 14.53 | 9.05 ± 0.73 b | 27.66 ± 0.77 b | 142.16 ± 8.37a | 80.55 ± 2.17a | 3.16 ± 0.86 | 297.75 ± 11.23 | 2.47 ± 0.75 |
| B | 11 | 49.48 ± 18.31 | 6.98 ± 0.14 c | 20.04 ± 1.89 c | 148.73 ± 3.09a | 83.24 ± 4.23a | 2.93 ± 0.21 | 318.47 ± 20.65 | 2.85 ± 0.19 |
| C | 18 | 46.28 ± 11.37 | 12.76 ± 0.60 | 36.73 ± 1.41 | 123.15 ± 2.46 | 68.11 ± 3.66 | 3.48 ± 1.16 | 268.16 ± 15.23 | 1.03 ± 0.11 |

A- mild anaemia; B- severe anaemia; C -normal controls; SBP- systolic blood pressure; DBP- diastolic blood pressure; Hb-haemoglobin; Hct- haematocrit; CRP- C-reactive protein. Comparison between groups A-C and B-C unpaired Student's t-test (a- $P < 0.05$; b- $P < 0.01$; c- $P < 0.001$). Data is presented as means ± SD

Table 2. Comparison of the cerebrovascular reactivity in groups A, B, C

| Group of study | A | B | C | A-B | A-C | B-C | A-B-C |
|-----------------------|--------------|--------------|--------------|------------|-------------|-------------|------------|
| RMCA | | | | | | | |
| MFV at rest | 48.78 ± 2.62 | 46.42 ± 3.49 | 54.88 ± 3.53 | NS | NS | NS | NS |
| MFV increase (cm/sec) | 6.84 ± 1.56 | 3.86 ± 1.13 | 13.05 ± 1.38 | $P < 0.05$ | $P < 0.001$ | $P < 0.001$ | $P < 0.01$ |
| MFV increase (%) | 12.79 ± 1.09 | 6.55 ± 0.67 | 23.25 ± 5.38 | $P < 0.01$ | $P < 0.001$ | $P < 0.001$ | $P < 0.01$ |

MFV- mean flow velocity; RMCA- right middle cerebral artery; AB, AC, BC- unpaired Student's t-test; comparison between groups ABC- one way ANOVA

Relationship CVR-anaemia

The correlation analysis (Pearson) between the percent increase in the MFV and the values of Hb in patients with anaemia is presented in Figure 1. There

was a very significant direct correlation between the two parameters ($r = 0.94$; $P < 0.001$). Moreover, the percent increase in the MFV correlated directly significantly with the values of Hct ($r = 0.85$; $P < 0.0001$).

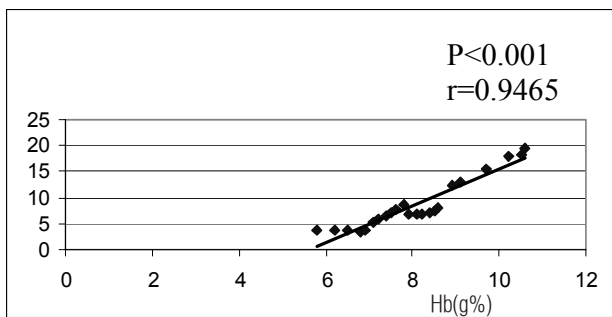


Figure 1. Correlation between haemoglobin values and the cerebrovascular reactivity in groups A and B (Pearson's test) MFV- mean flow velocity; Hb- haemoglobin

DISCUSSION

To date, the influence of anaemia on cardiovascular function is well documented. Non-haemodynamic and haemodynamic mechanisms are involved in chronic renal failure anaemia, aiming to compensate for a decrease in haemoglobin concentrations. Non-haemodynamic mechanisms include increased erythropoietin production to stimulate erythropoiesis, and increased oxygen extraction.¹⁶

In resting conditions, the non-haemodynamic factors and decreased affinity between oxygen and haemoglobin are capable of compensating for haemoglobin deficit. By contrast, when haemoglobin reaches a critical level (i.e., below 10 g/dl) or during non-resting circumstances, non-haemodynamic mechanisms are superseded by haemodynamic mechanisms.¹⁶

Amongst mechanisms of haemodynamic compensation, the decrease in vascular resistance is one of the most important factors, which represents the direct consequence of a reduction in blood viscosity and arterial dilation. Both aspects are related to the severity of anaemia, which is the case with CRF patients and secondary anaemia. In order to explain arterial vasodilation, two hypotheses have been forwarded: *hypoxic vasodilation*- due to hypoxia-generated metabolites, and *flow-mediated vasodilation*- due to increased blood flow, an effect that is mediated by endothelial cells and endothelium-derived relaxing factors.¹⁷

These adaptative mechanisms to anaemia have been proved to be the haemodynamic response of the cerebral vessels to chronic hypoxia in patients with CRF and secondary anaemia. Studies conducted on these patients have underlined the increase in *cerebral blood flow* in uraemic patients due to associated anaemia.¹⁸ Using the Xe-133 inhalation method, Vorstrup et al reported an increase in cerebral blood flow in anaemic patients on long-term haemodialysis.¹⁹

Changes in cerebral haemodynamics have been substantiated by studies with PET (O-15 H₂O-PET) which showed a significant increase in cerebral blood flow in CRF patients with severe anaemia that can be explained by brain tissue hypoxia due to insufficient O₂ delivery to the brain and by a reduction in blood viscosity.^{4,20}

More recently, attention has focused on the *cerebrovascular reactivity* in anaemic patients with CRF. Based on previous studies, attempts have been made in order to clarify the major importance of assessing the CVR in this category of patients. Early research concerning cerebral haemodynamics in CRF patients with anaemia has shown that the cerebrovascular response is decreased according to the level of haemodilution.²¹

Similar changes have been described by Kuwabara et al in their study conducted on CRF patients with anaemia in whom they demonstrated that the CO₂ response decreased in both mild and severe anaemic patients with CRF, and closely correlated with the severity of anaemia.⁴

Similar results have been reported by a study carried out in CRF children on conservative treatment, CAPD and HD, in whom the impaired cerebrovascular response (inappropriate decline in CBF in the MCAs to PCO₂ decrease) was documented by TCD after spontaneous hyperventilation for 30 seconds. The authors conclude that these modifications may be related to the stage of CRF (more prominent changes in CAPD and HD patients) and to impaired autoregulation of cerebral blood flow.¹⁵ HD may also affect the cerebral structure and haemodynamics.⁴

Our study reveals normal values of the basal flow velocity in both MCAs and an appropriate increase in the mean flow velocity during hypercapnia induced by the BHT, a fact that correlated significantly with the degree of anaemia.

Several mechanisms have been claimed to be instrumental in the impaired cerebrovascular response in CRF patients with anaemia. It has been postulated that resistant vessels have already been dilated fully by the brain tissue hypoxia induced by long-standing anaemia, and thus they are unable to dilate any further extent with the hypercapnia stimuli.⁴

Other mechanisms of impaired CVR have been cited, but are yet to be proved. It has been suggested that chronic hypoxia causes structural alterations of the cerebral vessels and that hypoxia-induced organic brain damage (white matter lesions) may reduce the cerebral vasodilatory capacity in CRF patients with anaemia. Moreover, a high CBF increases the delivery of uraemic toxins to the brain (including advanced glycation end-products, which enhance the oxidative

stress), with subsequent brain tissue damage.⁴

Of interest, brain oxygen metabolism is depressed in patients with CRF on or before the start of HD. The major cause for this condition is assumed to be either dysregulation of cerebral circulation or lower brain cell activity.²²

It is worth mentioning that hypertension was also considered in CRF patients with anaemia when analysing the conducive factors to impaired CVR. It is well known that hypertension may produce arteriosclerosis, thus leading to a decline in the compliance and dilation capacity of the vessel walls, including the cerebral vessels. Blood pressure levels, however, did not differ significantly in our patients with mild and severe anaemia, respectively.

Therefore, we assume that the potential consequences of hypertension on the CVR did not interfere with the interpretation of the BHT data. The same conclusion was reached in the study of Kuwabara et al, who found no significant differences concerning blood pressure levels between pre-dialysis-, HD-patients, and normal controls.⁴ In addition, other factors involved in arteriosclerosis, such as age, inflammation and malnutrition, which could have interfered with the ability of the cerebral vessels to respond to hypercapnia, did not show significant differences amongst the CRF patients and normal controls.

In our normal control group, the basal flow velocity was within normal values and the cerebrovascular response to hypercapnia was appropriate, as documented by the TCD measurements during the BHT. This is accordance with data reported by several authors in normal subjects during the BHT, in whom they revealed a percent increase in the MFV of 15-35% in both MCAs.^{5,10,12,23}

From the practical standpoint it should be underlined that the BHT is a useful tool in the assessment of the CVR by TCD, and easy to perform by comparison with the CO₂ test or the acetazolamide test.³ It has an excellent clinical tolerance, a fact observed in our patients too. The only adverse effect recorded in three patients was a compelling urge to breath by the end of the apneic phase.

In addition, several sources of error may occur, of which the fact that PaCO₂ may rise at different rates during the BHT in different subjects seems to be the most remarkable.³ However, functional information about the impaired cerebral autoregulation obtained with the BHT is of major import not only in CRF patients with anaemia, but also in patients with stenoses and occlusions in the carotid arteries,^{8,10,12,13} in diabetic patients with diabetic microangiopathy

within the cerebral vessels,^{24,25} and in post- menopausal women.²³

A highly impaired CVR, such as that documented in our CRF patients with severe anaemia (exhausted CVR) is of predictive value in identifying patients at increased risk of developing stroke during the follow-up period.

Correction of anaemia with recombinant human erythropoetin leads to a significant improvement in the CVR.²⁰

In conclusion, the CVR is impaired in patients with CRF and secondary anaemia. The cerebral vasodilatory capacity is diminished during hypercapnia induced by the breath- holding test. These cerebral haemodynamic changes correlate significantly with the severity of anaemia. Chronic hypoxia related to renal anaemia might play a role in the CVR impairment during hypercapnia. TCD proved to be a reliable tool in the assessment of CVR in these patients. Therefore, it should be performed in selected cases in order to detect patients with impaired CVR in due course, and subsequently to reduce the risk of stroke.

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