

# Hyperhomocysteinemia in preeclampsia is associated to higher risk pressure profiles

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**Abstract.** – Homocysteine levels have been determined with Chromatography on HPLC column, between the 20<sup>th</sup> and the 24<sup>th</sup> week of pregnancy, in women with analogous characteristics (a) normotensive, (b) with pregnancy-induced hypertension (PIH), low (LR), medium (MR), high risk (HR). The group they belonged to was confirmed after natural or caesarean delivery. All the patients were submitted to 24 hour blood pressure monitoring for the evaluation of further pressure risk parameters: mean arterial pressure (MAP), non dippers, percentages of pressure peaks.

Homocysteine levels in normotensive pregnant women ( $5.8 \pm 1.7 \mu\text{M}$ ) were low. Significant high levels of homocysteine were present proportionally to the risk degree of PIH. Higher levels of homocysteine statistically significant were present in non dippers of all groups (MR  $p < 0.05$ ; HR  $p < 0.01$ ).

A direct correlation between plasmatic homocysteine levels and pressure profiles was found out in non dippers ( $r = 0.56$ ,  $r = 0.55$ ,  $r = 0.50$  respectively) and in dippers ( $r = 0.7$ ,  $r = 0.75$ ,  $r = 0.60$  respectively), and also between levels of homocysteine, MAP value, and pathological percentages of systolic and diastolic nocturnal peaks. In pregnant women presenting preeclampsia afterwards, high levels of homocysteine were not different from mean values present in high risk PIH pregnant women ( $13.3 \pm 1.9$  vs.  $16.4 \pm 1.7 \mu\text{M}$ ).

High levels of homocysteine early determined in the second trimester of PIH pregnancies seem to be associated to a pregnancy higher risk, coexisting with dangerous pressure profiles. High levels confirm a pregnant woman to belong to a higher or lower risk degree of vascular damage, but in the same group context high levels of homocysteine do not allow to identify those pregnant women who will develop eclampsia.

*Key Words:*

Homocysteine, Preeclampsia, Ambulatory blood pressure monitoring.

## Introduction

Eclampsia is still at present also a dreadful complication of pregnancy-induced hypertension (PIH) and it is cause of high maternal-foetal mortality and morbidity. The consideration that under this condition anatomy functional alterations may establish before clinic manifestations justifies a growing interest in the pathogenetic processes underlying, as well as in the identification of predictive factors to recognize higher risk patients in early pregnancy<sup>1-5</sup>. Among the predictors, the evidence of high pressure values still is the most reliable<sup>6-14</sup>. Among biohumoral parameters, homocysteine has recently been considered a possible remarkable cause of vascular damage<sup>15-18</sup>. Some studies suggest that hyperhomocysteinemia, already noticed in early pregnancy, may constitute a precocious marker of maternal-foetal risk for eclampsia<sup>19-22</sup>.

In our study we wanted to verify whether the early determined highest levels of homocysteine, in PIH pregnant women, coexist with pressure profiles considered particularly at risk, and whether, consequently, this association may be predictive of eclampsia onset.

## Material and Methods

We have selected a survey of pregnant women, observed and supervised in this last year, coming from Obstetric Departments and from the Territory for routine check-ups, or for suspicious arterial hypertension (data isolated by a family doctor), or for manifested PIH, or for the coexistence of risk factors

(hypertensive familiarity, former gestosis, diabetes, etc.).

After carrying out clinic checks, all anamnestic data were collected. All patients, were observed between the 20<sup>th</sup> and the 24<sup>th</sup> week of pregnancy; in this period they were submitted to basal arterial pressure measurement (3 measurements at regular intervals of 30 minutes) and to Ambulatory Blood Pressure Monitoring (ABPM) with Takeda TM2430 instruments, measuring arterial pressure every 15 minutes during the day and every 30 minutes at night.

All patients were also submitted to computerized ECG (HP4760 A Cardiograph), to Doppler umbilical artery velocimetry at the right moment, and, in "at risk" cases, an echo Doppler of supra-aortic vessels (ATL 800 10Hz probe) and a cardiac Holter (Cardioscan 10). Bio-humoral checks (hematic crisis, hepatic and renal function, coagulative and metabolic indexes, etc.) were made in order to point out possible risk factors or/and pathologic conditions.

According to the results of checks, all patients were subdivided into 4 groups: (1) normotensive pregnant women (NP), without clinic-anamnestic risk factors; (2) low risk pregnancy induced hypertension (PIH) pregnant women (LR); (3) medium risk PIH pregnant women (MR); (4) high risk PIH pregnant women (HR); in this group 5 patients developed eclampsia between the 30<sup>th</sup> and the 32<sup>nd</sup> week of pregnancy.

This classification adopted by our study group is the one codified and established by the International, European and Italian Association of Arterial Hypertension, by the Gynaecologic and Obstetric Society and by the WHO, with reference to pressure data and to the coexistence of other risk factors<sup>23-25</sup>.

In pregnancy hypertension is usually defined when pressure levels are over 140/90 mmHg, or when in the first three months the systolic pressure increases  $\geq 25$  mmHg and/or the diastolic pressure increases  $\geq 15$  mmHg, compared to the values before pregnancy. Pregnant women have been considered at "low risk" of preeclampsia if other risk factors (previous gestosis, cardiovascular diseases, renal diseases, metabolic disease, etc.) are not associated to hypertension. Pregnant women have been considered at medium or high risk when pressure values are progres-

sively higher (up to 170/110 mmHg) and/or when the mentioned risk factors coexist.

Between the 20<sup>th</sup> and the 24<sup>th</sup> week of gestation a 5 ml of peripheral blood sample was taken from normotensive pregnant women and from those with PIH, after their informed consent. The samples, kept in cooled-down polyethylene test tubes, containing EDTA (5  $\mu$ M/ml of blood), were immediately put into a freezer at  $-20^{\circ}$ , later to be used in dosing homocysteine.

Unlike other authors<sup>19-21</sup> who took the blood samples between the 10<sup>th</sup> and the 15<sup>th</sup> week of pregnancy, it was considered a consistent choice taking the samples between the 20<sup>th</sup> and the 24<sup>th</sup> week, since pathogenetic alterations causing preeclampsia may probably occur in this period.

Subsequently, twelve normotensive pregnant women were selected to match PIH pregnant women with respect to anthropometric data (i.e., age, BMI, diet, etc.); in addition, the selected normotensive pregnant women were found to be free from any risk factor.

The value of arterial pressure was pointed out at the moment of the observation (20<sup>th</sup>-24<sup>th</sup> week), finding it useful to report the value of the MAP (mean arterial pressure) (systolic pressure + 2/3 diastolic pressure /3) as average  $\pm$  SD, as well as the value of some parameters derived from 24-hour ambulatory blood pressure monitoring: percentage of pressure peaks, nocturnal and diurnal pressure variability, dipper and non dipper pattern. These parameters are more significant in the prediction of unfavourable outcome than others, according to what emerged from Societies and Study Groups<sup>9,14,26</sup>. The dipper/non dipper behaviour is given a) by the nocturnal reduction of blood pressure over 10% compared to diurnal pressure (dippers); b) by a nocturnal reduction less than 10% compared to diurnal pressure (non dippers). A number of studies point out that non dippers show a higher incidence of organ damage and cardiovascular events compared to dippers. It is also widely documented organ damage (ventricular hypertrophy, cerebrovascular lacunas, micro-albuminuria, carotid intima-media thickness, atherosclerotic plaques) is correlated more to mean systolic/diastolic arterial pressure in 24hrs, than to correspondent values obtained with routine methods<sup>27</sup>.

No one of the patients had taken any medicine from the beginning of pregnancy up to the moment of the first observation and of the blood taking. After the identification and after the blood taking, the group at risk was treated with Alpha-Metildopa (from 250 mg to 1000 mg/die); in some of more severe clinic cases (high pressure levels, initial organ damage, etc.), Nifedipin (from 20 mg to 60 mg/die) was given instead, or added.

Routine bio-humoral parameters (coagulation, hepatic and renal function, hemochrome) were carried out with common methods.

The dosage of plasma homocysteine was carried out with liquid phase high resolution Chromatography on HPLC column (Polytechne-Livorno Italy reagents).

## Statistics

Results are expressed as means  $\pm$  SD (standard deviation).

In groups the Student's t test method was applied and the p statistic calculus was evaluated.

The curves of correlation between plasma levels of homocysteine and the percentages of systolic and diastolic nocturnal peaks and MAP values, were calculated adopting Pearson's correlation coefficient.

## Results

### *Plasma Homocysteine*

Data concerning our study are reported on Table I.

In normotensive pregnant women (NP) a mean value of  $5.8 \pm 1.7 \mu\text{M}$  was obtained; in low-risk (LR)  $7.2 \pm 1.5 \mu\text{M}$  (statistically with low relevance  $p < 0.05$ ): both the two groups were made of dipper patients (Figure 1).

Both the high risk group (HR) and the medium risk group (MR) showed an increased plasma concentration of homocysteine  $16.4 \pm 5.3$  and  $9.5 \pm 2.1 \mu\text{M}$  average levels, respectively. These value are statistically significant ( $p < 0.001$ ) compared to those of LR and NP.

Subdividing HR and MR into subgroups of non dippers and dippers, the plasma level of homocysteine was higher in non dippers than in dippers ( $p < 0.001$ ) in the HR group had  $19 \pm 5.2$  vs.  $12.5 \pm 2.1 \mu\text{M}$  ( $p < 0.01$ ); the MR group  $11.3 \pm 2.3$  vs.  $8.5 \pm 1.2 \mu\text{M}$  ( $p < 0.05$ ). Within the group of HR pregnant women plasma homocysteine levels in the five women who had eclampsia were:  $13.3 \pm 1.9 \mu\text{M}$  vs  $16.4 \pm 1.7 \mu\text{M}$ , statistically with low relevance ( $p < 0.05$ ).

### *MAP and Pressure Variability*

MAP in NP and LR was 47.75 vs. 51.76 mmHg ( $p < 0.01$ ) (Table I). In HR and MR pregnant women, subdivided into non dippers and dippers, the results were: HR 75 vs. 71.12 ( $p < 0.001$ ), MR 68.71 vs. 65.76 mmHg ( $p < 0.01$ ). MAP values correlated well with homocysteine levels in PIH pregnant women non dippers, who belonged to both HR and MR groups.

As for the percentage of pressure peaks (expressing pressure variability), values over 10-12%, especially nocturnal ones, are considered pathologic<sup>26</sup>. A statistically significant difference ( $p < 0.001$ ) was pointed out between non dippers and dippers with over 11% systolic and diastolic nocturnal peaks both in HR and MR groups. PIH pregnant women were subdivided into dippers and non dippers, not considering the group they belonged to (HR or MR). In each subgroup we wanted to verify the correlations between homocysteine levels and the following pressure parameters: percentage of systolic nocturnal (S/N) peaks, percentage of diastolic nocturnal (D/N) peaks, MAP values.

The results were:

- in dippers, homocysteine vs. percentage of S/N peaks  $r = 0.56$ ; homocysteine vs. percentage of D/N peaks  $r = 0.55$ ; homocysteine vs MAP  $r = 0.50$ .
- in non dippers, homocysteine vs percentage of S/N peaks  $r = 0.7$ ; homocysteine vs. percentage of D/N peaks  $r = 0.75$ ; homocysteine vs MAP  $r = 0.60$ .

## Discussion

These data suggest that plasma homocysteine concentration in normotensive preg-

**Table 1.** Clinical and pressure parameters (valued also with ABPM method) in groups of pregnant women: NP = normotensive; LR = low risk PIH pregnant women; MR = medium risk PIH pregnant women; HR = high risk PIH pregnant women.

	NP (n° 12)	LR (n° 13)	MR (n° 20)	HR (n° 20)
Age (years)	23.5 ± 3.7	22.8 ± 2.4	24.2 ± 3.1	23.6 ± 6.2
Primiparas	5 (41.6%)	7 (53.8%)	11 (55%)	14 (70%)
Week of pregnancy	23 ± 1	22 ± 2	22 ± 1	22 ± 2
BMI (kg/m <sup>2</sup> )	26.6 ± 1.8	26.1 ± 1.4	26.4 ± 1.9	26.8 ± 2.2
SBP (mmHg)	112.4 ± 10.2	138.6 ± 6.6	152.2 ± 4.8	162.6 ± 9.2
DBP (mmHg)	58.2 ± 2.4	64.4 ± 8.2	76.2 ± 10.8	94.6 ± 9.7
MAP (mmHg)	47.7 ± 3.2*	51.7 ± 1.1*	D 65.7 ± 1.5** ND 68.7 ± 1.1**	71.1 ± 1.3*** 75 ± 1.3***
Nocturnal systolic peaks %	2.17 ± 1.03*	5.6 ± 1.1*	D 10.1 ± 1.5** ND 9.6 ± 1**	14 ± 4.4*** 20.5 ± 4.9***
Nocturnal diastolic peaks %	2.2 ± 1.2*	7 ± 1.5*	D 10.8 ± 1** ND 12.2 ± 1.5**	13.1 ± 4.3*** 20.7 ± 4.4***

\*, \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

BMI (Body Mass Index); SBP (Systolic Blood Pressure); DBP (Diastolic Blood Pressure); MAP (Mean Arterial Pressure); D = dippers; ND = non dippers.

nant women are lower compared to non pregnant women of the same age. The mechanism responsible for this decrease<sup>19,22,28,32</sup> is unknown, but it may involve maternal-foetal metabolism of the placenta or of the liver. This metabolic intervention might have “protective” finality, without which homocysteine levels would probably be higher<sup>19,21,32</sup>. Homocysteine levels, which are considered within normal limits (< 15  $\mu\text{M}$ ) in non pregnant women, are considered at risk in pregnancy<sup>21</sup>. It is proved that in the complex biochemical mechanism of methionine-homocysteine high levels of homocysteine may depend, even transitorily, on lack of precursors (folates, B<sub>12</sub>, etc.) or on metabolic enzyme alterations<sup>33-36</sup>.

In women with preeclampsia the enzyme N5 N10 MTHFR (methylene-tetra-hydro-folate reductase), which determines de-/di-methylation, has been intensely studied<sup>37</sup>. Genetic alterations have been found<sup>33-35</sup> which are associated to high levels of homocysteine quite early (15<sup>th</sup> week) with clearly predictive meaning.

Other studies<sup>36</sup> do not confirm the alterations of MTHFR, but that would confirm hyperhomocysteinemia may also occur owing to other mechanisms.

Recently it has been found higher levels of homocysteine in early pregnant women (10<sup>th</sup>-

15<sup>th</sup> week) who would later develop preeclampsia<sup>20,22,29,31</sup>. Only few Author has not found significant variations in homocysteine levels thus denying predictive meaning to hyperhomocysteinemia<sup>28,30,37</sup>.

Raymarkers et al<sup>36</sup> found that in eclampsia the levels of homocysteine are higher than in normotensive pregnant women, but those levels are similar or not higher than those verified in non pregnant healthy women. The same levels are not correlated to alterations of T677 genotype of MTHFR gene.

From our data it is confirmed that pregnant women developing preeclampsia showed hyperhomocysteinemia already at 20<sup>th</sup>-24<sup>th</sup> week; it also appears that higher levels of homocysteine, whatever their origin (metabolic enzyme alterations and/or lack of precursors), are present in pregnant women with a higher risk of preeclampsia; the risk is evaluated by other clinic anamnestic parameters, among them one of the most important is the pressure parameter<sup>6-8</sup>.

From our data, in fact, they seem to be correlated to pressure parameters (MAP and pressure variability), of which the meaning of risk is at present widely proved<sup>11-14</sup>.

Several studies have conjectured the existence of endothelial dysfunction in the pathogenesis of eclampsia. During eclampsia high homocysteine levels might induce vascular

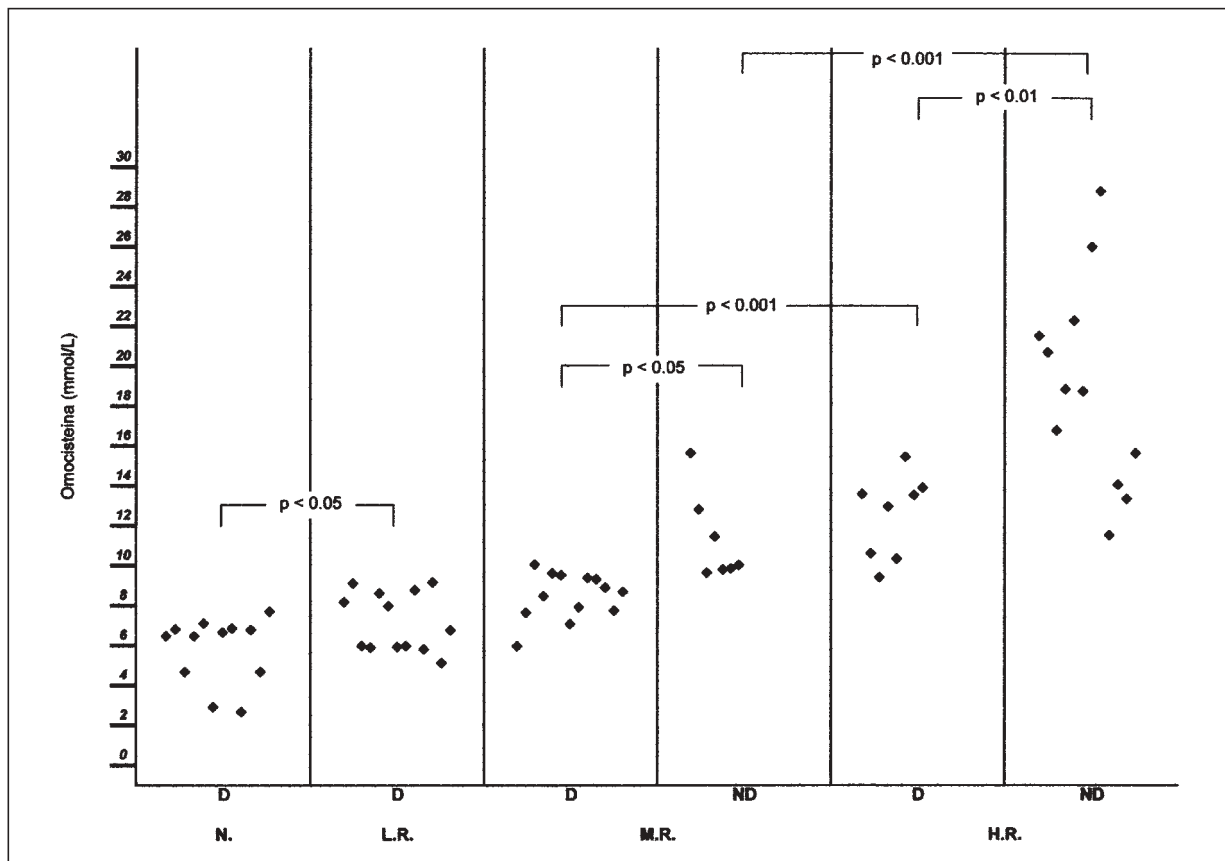


Figure 1. Levels of homocysteine ( $\mu\text{M/l}$ ) indicated also as average values  $\pm$  SD, observed between the 20<sup>th</sup> and the 24<sup>th</sup> week of pregnancy in groups of pregnant women. NP = normotensive; LR = low risk PIH pregnant women; MR = medium risk PIH pregnant women; HR = high risk PIH pregnant women. Among the HR and the MR groups a subdivision was also made based on ABPM data between D = dippers and ND = non dippers. Statistical significances are noticed in the groups.

damage through an endothelial dysfunction caused by oxidative stress<sup>1,21,38-40</sup>.

In conclusion, from our data it appears that low levels of homocysteine are present in normal pregnancy. Levels of homocysteine, which may be considered “normal” in non pregnant healthy women, might be not tolerated in pregnancy, in fact, progressively higher plasmatic levels of homocysteine are associated to a higher risk of preeclampsia, as they coexist with dangerous pressure profiles (MAP, percentage of pressure peaks, nocturnal and diurnal pressure variability, dipper and non dipper pattern). Probably high levels of homocysteine may be considered a precocious marker of vascular damage and may denote a higher risk of preeclampsia. Within the high risk group, at any rate, hyperhomocysteine is not able to

“predict” which pregnant women will develop eclampsia.

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