UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
PhD STUDIES SCHOOL

PhD THESIS

- ABSTRACT -

‘ SEARCHING THE MISSING LINK BETWEEN ALZHEIMER’S DISEASE AND TYPE 2 DIABETES’

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Contents

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1. Literature Review

1.1 Peripheral insulin resistance

Peripheral insulin resistance (IR) is defined as an inadequate response by insulin target tissues (skeletal muscle, liver, and adipose tissue) to the physiologic effects of circulating insulin.

Although IR not always leads to the development of type 2 diabetes (T2D), this is one of the central mechanisms involved; T2D appears when insulin-resistant individuals cannot secrete the increased amounts of insulin needed to compensate for the IR. Over the years have been introduced many methods of evaluating peripheral IR, including anthropometrical and biological markers. However, the method most used when assessing IR is the homeostasis model assessment of insulin resistance (HOMA-IR).

1.2 Alzheimer's disease – 'insulin resistant brain state'

Alzheimer's disease (AD) is the most common cause of dementia and it accounts for 70% to 90% of all forms dementia. Over the past several decades, the prevalence rates of sporadic AD have augmented, even after correcting for increasing longevity. Growing evidence supports the concept that AD is fundamentally a metabolic disease that results in progressive impairment in the capacity of the brain to utilize glucose and respond to insulin and insulin-like growth factor (IGF) stimulation. This glucose metabolism impairment associated with AD justified the term 'type 3 diabetes' which accurately reflects the fact that AD represents a form of diabetes that selectively involves the brain and has molecular and biochemical features that overlap with both type 1 diabetes and T2D. Although it seems that AD is indeed 'type 3 diabetes', many aspects of the linking mechanisms between AD and T2D remain to be discovered. Recent studies have focused on the importance of adipokines in this process, the most studied being adiponectin, leptin and progranulin (PGRN).

1.3 Progranulin and its involvement in both peripheral insulin resistance and Alzheimer's disease

Recently recognized as an adipokine, PGRN is mentioned for the first time in relationship to peripheral IR by Youn et al. in 2009. Since then, a number of studies reconfirmed the role of PGRN in IR, obesity and T2D.

Regarding AD, although studies have proposed this protein as a biological marker of this form of neurodegeneration, its role in the development of this disease is still unclear and controversial.

2. Personal Contributions

2.1 Aims of the study

We propose a pilot study which evaluates the serum PGRN levels and the possible contribution of this protein to the association between AD and T2D. We also propose a study of peripheral IR evaluated using classical and newly introduced markers of IR, as well as the relationship between IR measured using classical parameters and the levels of serum PGRN. The aims of our study were:

- To study peripheral IR in the study population using classical and relatively new markers of IR
- To evaluate the usefulness of anthropometrical and biological markers of IR in an elderly population.
- To evaluate whether PGRN is useful as a marker of IR in an elderly population.
- The quantitative determination of serum PGRN levels in subjects with AD, T2D, subjects with both pathologies and a control group and the comparison of the found results between the 4 groups.

2.2 Material and methods

The study was conducted during PhD external internship, between September 2012 and May 2013. The subjects enrolled in the study were recruited from the Endocrinology and Diabetology and Neurology Out-patient Departments of Campus Bio-Medico University of Rome. The study was approved by the ethics committee of the University and all the participants have read and signed the informed consent.

We conducted a pilot study on 40 subjects, as described: Group 1: 10 subjects with both AD and T2D; Group 2: 10 subjects with AD without T2D; Group 3: 10 subjects with T2D without AD; Group 4: 10 healthy controls age and sex matched.

All the subjects included in the study met the following inclusion criteria: age > 60 years; the duration of T2D > 1 year; the subjects without AD scored at least 27 on the mini mental state evaluation (MMSE). We also established exclusion criteria: anti-inflammatory treatment; corticosteroid therapy; estimated glomerular filtration rate (eGFR) calculated using CKD-EPI formula<30mL/mim/1.73m^2; hepatic disease; recent infection/inflammation/acute disease; history of cancer; subjects with T2D that receive insulin; in the subjects with only AD and in the control group the diagnosis of T2D was excluded after performing both blood fasting glycemia and glycated haemoglobin (HbA1c) that had values within the normal range; subjects with only T2D and controls that have scored less than 27 at MMSE.

The collected data included: demographic data (gender, age); anthropometric data (weight, height, waist circumference (WC), hip circumference, wrist circumference); current treatment;

cognition tests (MMSE); laboratory exams (HbA1c, fasting plasma glucose (FPG), fasting plasma insulinemia, fasting C peptide, total Cholesterol, HDL Cholesterol, triglycerides (TG), azotemia, creatinine, microalbuminuria, transaminases, high sensitive C reactive protein (hsCRP), complete blood count).

The parameters associated with IR were evaluated: HOMA-IR, body mass index (BMI), WC, wrist circumference, waist to hip ratio (WHR), waist to height ratio (WHtR), body adiposity index (BAI), TG/HDL ratio, visceral adiposity index (VAI), C peptide ratio which equals 20/(fasting C peptide (nmol/L) X FPG (mmol/L)). Two special assays, plasma PGRN and apolipoprotein E (Apo E) genetic analysis, were also performed.

Recorded data were analyzed using the Statistical Package for the Social Sciences (SPSS) 17.00 software (IBM Corporation, Armonk, NY, USA).

2.3 Markers of peripheral insulin resistance in the study population

2.3.1 Results

We evaluated IR using a classical parameter, HOMA-IR, but also anthropometrical and biological parameters that scientific literature reported as markers of IR, and found statistically significant differences between the 4 study groups regarding WC (p=0.007), BMI (p=0.021), BAI (p=0.005), WHR (p=0.025), WHtR (p=0.004), C peptide ratio (p=0.002) and hsCRP (p=0.032). It was observed that even though HOMA-IR was not statistically significant different between the 4 groups, however it had higher values in the subjects with T2D and in those with both T2D and AD. Furthermore, all the other parameters investigated in which we did not find a statistically significant difference showed the same trend.

For a better understanding of these parameters and their importance as markers of IR, the study population was regrouped according to the presence of T2D into 2 groups. We observed that HOMA-IR (p=0.049) as well as all the anthropometrical parameters were statistically significant higher in the subjects with T2D.

Correlation analysis between HOMA-IR and all the others studied parameters were performed both for the entire population and in the subjects with T2D. Statistically significant results are shown in table 2.1.

Table 2.1. Significant correlations between HOMA-IR and anthropometrical and biological markers of IR in the entire study population and in the subjects with T2D.

Studied parameter	Correlation p value	
	Entire study population (n=40)	Subjects with T2D (n=20)
WC (cm)	**<0.001**	**0.014**
Wrist circumference (cm)	**0.033**	NS
BMI (Kg/m^2)	**0.001**	NS
BAI (%)	**0.002**	NS
WHR	**0.025**	NS
WHtR	**0.001**	NS
Fasting C peptide (ng/mL)	**<0.001**	**0.001**
C peptide ratio	**<0.001**	**0.001**

NS not significantly statistic

The analysis of the Area under the ROC curve was used to evaluate the usefulness of the studied anthropometrical and biological markers as independent predictors of IR (evaluated by HOMA-IR) both in the entire population and in the subjects with T2D. In our study, WC (area=0.755, p=0.017), BMI (area=0.780, p=0.009), BAI (area=0.760, p=0.015), WHtR (area=0.755, p=0.017), C peptide (area=0.943, p<0.001) and C peptide ratio (area=0.912, p<001) were such independent predictors when we analyzed the entire study population. It was observed that higher values of WC, BMI, BAI, WHtR and C peptide are independent predictors of IR, while lower C peptide ratio independently predicts the risk of IR. The analysis of the Area under the ROC curve for C peptide ratio, C peptide and WC as predictors of IR evaluated by HOMA-IR in the subjects with T2D showed that only C peptide (area=0.934, p=0.002) and C peptide ratio (area=0.923, p=0.002) were independent predictors of IR. However, while higher levels of C peptide are predictive of IR, when considering C peptide ratio, its lower values are independent predictors of IR.

Wrist circumference, anthropometrical parameter recently associated with IR, was analyzed in relationship to all the others studied parameters. As we have previously mentioned above, wrist circumference was correlated with HOMA-IR, but was not an independent predictor of IR. Table 2.2 presents the significant correlations between wrist circumference and the others anthropometrical and biological parameters examined. The analysis was performed both for the entire study population and for the subjects with T2D.

Table 2.2. Correlations between wrist circumference and the others anthropometrical and biological markers of IR in the entire study population and in the subjects with T2D

Studied parameter	Correlation p value	
	Entire study population (n=40)	Subjects with T2D (n=20)
WC (cm)	**<0.001**	**0.018**
BMI (Kg/m^2)	**<0.001**	**<0.001**
BAI (%)	**0.013**	NS
WHR	**0.003**	NS
WHtR	**0.002**	NS
Fasting C peptide (ng/mL)	**<0.001**	**0.003**
C peptide ratio	**0.004**	NS

*negative correlation NS not significantly statistic

The relationship between the markers of IR and chronic kidney disease (CKD) was also evaluated, as IR, obesity and T2D are risk factors for CKD. Therefore, we analyzed the correlations between eGFR calculated using CKD-EPI formula and all the parameters previously discussed in the entire population and found significant negative correlations between eGFR and wrist circumference (p=0.017), fasting C peptide (p=0.007), TG/HDL (p=0.008) and VAI (p=0.011). The analysis of the Area under the ROC curve showed that all the parameters correlated to eGFR were also independent predictors of CKD, defined as eGFR<60mL/min/1.73m^2. Furthermore, in the subjects with T2D, eGFR was negatively correlated with wrist circumference (p=0.009) and fasting C peptide (p=0.007).

2.3.2 Discussions

When interpreting the results of our study, first of all we must take into consideration that it was performed in an elderly population (mean age 74.6±4.9 years old) and on a small number of subjects. To our best knowledge, there was no previous study analyzing the usefulness of wrist circumference as a marker of IR in the elderly, although this measurement proved to be a significant predictor of diabetes and metabolic syndrome in an adult population. According to the results of our study, although wrist circumference was not an independent predictor of IR, it was correlated with the values of HOMA-IR. Regarding the biological parameters studied, only fasting C peptide and the newly described C peptide ratio were correlated with HOMA-IR both when we analyzed the entire study population and the subjects with T2D, and proved to be independent predictors of IR in both analysis.

An interesting finding of our study was the relationship between wrist circumference and CKD, proving that wrist circumference was an independent predictor of CKD.

2.4 Progranulin as a biological marker of peripheral insulin resistance

2.4.1 Results

Given the possible role played by PGRN in AD pathology, in order to avoid misinterpretations, we chose to evaluate the usefulness of PGRN and PGRN/kg as biological markers of IR in our population only in the subjects with T2D without AD vs. the control group. PGRN was analyzed in relationship with HOMA-IR and we found a statistically significant correlation between serum PGRN levels and HOMA-IR values ($p=0.043$) only in subjects with T2D (without AD). Furthermore, PGRN had higher levels in the IR subjects compared with subjects that had a HOMA-IR<2.6, but this difference was not statistically significant ($p=0.059$). We also compared the PGRN values between subjects with T2D and IR, non IR subjects with T2D and controls and we found a statistically significant difference between the 3 groups ($p=0.007$). The post-hoc analysis showed that this was given by the statistically significant higher values of PGRN in IR subjects with T2D compared to the control group ($p=0.005$).

A separate gender statistics of the serum PGRN showed there was no statistically significant difference between males and females. However we did find a statistically significant correlation between PGRN and HOMA-IR in females ($p=0.046$).

The only statistically significant result when we performed the same analysis for PGRN/kg found that PGRN/kg ratio was significantly statistic higher in females ($p=0.048$).

2.4.2 Discussions

As we have previously emphasized, when interpreting the results of our study we must also take into consideration that it was performed in an elderly population, while the majority of the studies regarding IR are performed in adult populations.

In our study, PGRN levels were correlated with HOMA-IR in the subjects with T2D. Furthermore, we also found a correlation between PGRN and HOMA-IR in the females included in the study.

2.5 Progranulin and its role in the association between Alzheimer's disease and type 2 diabetes

2.5.1 Results

We performed the analysis of serum PGRN levels in the studied groups. As PGRN proved to be an adipocitokyne, therefore being synthesized by fat cells, we also performed the PGRN/kg ratio. Table 2.3 presents the determined values in the 4 groups.

As PGRN values significantly deviated from a normal distribution, a logarithmic transformation was performed which led to a variable with normal distribution. After performing ANOVA test on the newly obtained values, a statistically significant difference was

observed between the 4 groups (p=0.01). However, the pos-hoc analysis showed that this difference is mainly due to the statistically significant difference between the subjects with both AD and T2D (group 1) and the subjects with T2D (group 3), proved by a p value of 0.006. Although not statistically significant (p=0.078), we also noted PGRN levels in the subjects with T2D were higher than in the control group.

The non-parametric Kruskal-Wallis (p=0.028) and Mann-Whitney tests, performed for the original values registered, also confirmed these findings.

Table 2.3. The values of PGRN and PGRN/kg in the study population (data presented as mean±SD)

Variable	AD+T2D n=10	AD n=10	T2D n=10	Controls n=10
PGRN (ng/mL)*	87.23±19.16	105.64±33.4	132.88±36.6	99±22.87
PGRN/kg*	1.21±0.29	1.7±0.5	1.65±0.53	1.51±0.5

* Variables significantly deviate from a normal distribution

Taking into account that the carriers of ε4 allele have a two-fold increased risk of developing AD, we also analyzed the relationship between PGRN levels and Apo E genotype, but we did not find statistically significant differences between subjects that have and the subjects in which the ε4 allele was absent.

PGRN/kg analysis in the study population was performed using non-parametric tests for the original values, as well as after logarithmical transformation to variables with normal distribution. Both Kruskal-Wallis and ANOVA test showed there was no statistically significant difference regarding PGRN/kg values between the 4 groups. However, we did find statistically significant differences between the subjects with both AD and T2D and the ones with AD (p=0.023), as well as the ones with T2D (p=0.049).

We find of interest the fact that although there was no significantly difference between PGRN serum levels between the subjects with both AD and T2D and the subjects with AD, when referring to PGRN/kg ratio, we found statistically significant higher values in the subjects with AD.

2.5.2 Discussions

When interpreting the results of ours study, we must take into consideration that these data come from a pilot study, trying to elucidate a small issue of the association between AD and T2D. To our knowledge, till the present date, all the studies that were performed in order to assess PGRN and its role in AD did not take into account the parameters of glycemic

metabolism or the presence of T2D. We find interesting that although PGRN was proposed as a marker of IR in subjects with T2D, when T2D was associated with AD, the levels of PGRN are statistically significant lower. However, the findings regarding PGRN in subjects with AD are comparable with what the scientific literature reports. Although at a first glance the results of our study might be surprising, we cannot overlook some recent studies that have demonstrated that both leptin and adiponectin, adipocytokines involved in IR, have the opposite effects when studied in subjects with AD.

3. Final Conclusions

- In our study, the newly introduced index of IR, C peptide ratio, proved to be the best independent predictor of IR (evaluated with HOMA-IR).
- Wrist circumference, an easily measurable anthropometrical parameter, uninfluenced by clothing or the subjects' post-prandial state, was a good marker of IR in an elderly population, making us suggest its inclusion in the clinical practice as an useful anthropometrical parameter when evaluating IR.
- C peptide ratio, TG/HDL, VAI and wrist circumference were independent predictors of CKD. However, as both TG/HDL and VAI are influenced by the lipid lowering treatment and C peptide ratio is relatively expensive to determine, we consider that wrist circumference might be introduced in the clinical practice as a parameter associated with CKD. However, as to our knowledge, this was the first study concerning the relationship between wrist circumference and CKD, larger cross-sectional and prospective studies are needed in order to confirm this findings and to determine appropriate gender-related cut-off values for this parameter.
- In our study, serum PGRN was correlated with HOMA-IR in elderly subjects with T2D. Taking into account our study, although on a small sample size, as well as the data existent in the scientific literature, we consider that serum PGRN is a good biological marker of IR.
- The statistically significant difference regarding the levels of circulating PGRN found between the subjects with AD and T2D and the ones only with T2D, as well as the differences of the PGRN/kg ratio between subjects with AD and T2D and the ones only with AD, and respectively T2D, make us believe that PGRN plays a role in the association of this two pathologies. However, as the reported results came from a small sized pilot study, future researches on an enlarged sample size paired with fundamental research studies might clarify the role and the mechanism through which PGRN, as well as other adipokines, influence this association.