Journal of Affective Disorders 234 (2018) 139–147

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

Alterations of the serum *N*-glycan profile in female patients with Major Depressive Disorder

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ARTICLE INFO

Keywords:
Childhood sexual abuse
Inflammation
Major Depressive Disorder
Serum *N*-glycan profile
DSA-FACE

ABSTRACT

Background: Glycans are short chains of saccharides linked to glycoproteins that are known to be involved in a wide range of inflammatory processes. As depression has been consistently associated with chronic low-grade inflammation, we asked whether patients with Major Depressive Disorder show alterations in the *N*-glycosylation pattern of serum proteins that might be linked to associated changes in inflammatory processes.

Methods: In a study cohort of 21 female patients with an acute depressive episode and 21 non-depressed female control subjects aged between 50 and 69 years, we analyzed the serum *N*-glycan profile by DNA Sequencer Adapted-Fluorophore Assisted Carbohydrate Electrophoresis (DSA-FACE) and assessed the serum levels of interleukin (IL) − 6, tumor necrosis factor (TNF)-α and C-reactive protein (CRP) by chemiluminescence immunoassays and nephelometry.

Results: Compared to controls, MDD patients showed significant differences in the serum levels of several *N*-glycan structures. Alterations in the serum *N*-glycan profile were associated with depressive symptom severity and exploratory analyses revealed that they were most pronounced in MDD patients with a history of childhood sexual abuse. Furthermore, MDD patients showed higher levels of IL-6 and a trend for higher CRP levels, which were also associated with similar alterations in the serum *N*-glycan profile as those characteristic for MDD patients.

Limitations: The relatively small sample size and the presence of potential confounders (e.g., BMI, smoking, medication).

Conclusion: The results offer the first evidence that specific differences in the *N*-glycosylation pattern of serum proteins constitute a so far unrecognized level of biological alterations that might be involved in the immune changes associated with MDD.

1. Introduction

Depression is a severe disease that affects more than 300 million individuals worldwide (World Health Organization, 2017). On a psychological level, experiences of chronic and traumatic stressors constitute major risk factors for the development of depression. In particular traumatic experiences during early life (e.g., childhood sexual abuse) seem to influence the course of the disease: individuals with a history of adverse early life experiences are not only at a higher risk to develop depression, but are also twice as likely to be resistant to antidepressant treatment (Nelson et al., 2017). Besides the typical depressive phenotype (e.g., fatigue, depressed mood, and difficulties concentrating), depressed individuals also often show increased behavioral risk patterns such as smoking, reduced physical activity, and a higher body-mass-index, which may be one reason for the higher frequency of physical diseases observed among depressed patients (De Hert et al., 2011). On a biological level, depression itself has, however, also been consistently associated with immune alterations and a phenotype of

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https://doi.org/10.1016/j.jad.2018.02.082
Received 20 October 2017; Received in revised form 1 February 2018; Accepted 25 February 2018
Available online 27 February 2018
0165-0327/

chronic low-grade inflammation (Berk et al., 2013) as indicated by increased serum levels of the pro-inflammatory cytokines interleukin (IL) − 6, tumor necrosis factor (TNF)-α, and C-reactive protein (CRP) (Dinan, 2009; Miller et al., 2009; Wolkowitz et al., 2010).

Almost all molecular processes that are critical for physiological immune functioning (e.g., cell-cell communication, signal transduction, host-pathogen interactions) involve glycoproteins (Ding et al., 2011; Comelli et al., 2006), i.e. proteins which are post-translationally modified through the controlled enzymatic attachment of complex oligosaccharide chains (glycans) to the oxygen atom of serine or threonine amino acid (*O*-glycosylation) or the nitrogen atom of asparagine amino acid (*N*-glycosylation) in the protein (Lauc et al., 2014). The attachment of different glycans to the same protein (resulting in different glycoforms) influences its biological function, as it has an impact on the conformation, solubility, antigenicity, and the recognition of the associated glycoprotein (Varki et al., 2009). Alterations in the protein glycosylation pattern thereby allow the organism to fastly adapt its biological functions to changing environments (Lauc et al., 2014). Besides environmental factors, protein glycosylation is further influenced by genetic factors such as gender (Ding et al., 2011) and by the physiological and biochemical status of the individual (Gornik and Lauc, 2008). For instance, inflammatory cytokines can regulate the expression of glycan modifying enzymes such as *α1,3-fucosyl-transferase, α2,3-sialyl-transferase*, and *N-acetylglucosaminyl-transferase* (Higai et al., 2005) and thereby influence the *N*-glycosylation pattern of immune proteins. Even minor modifications in the glycosylation pattern of immunoglobulin G (IgG), the most abundant glycoprotein in human serum (Debruyne et al., 2010), are, vice versa, associated with increased cytokine activity (Gornik and Lauc, 2008), which can further activate the immune system and exacerbate the pro-inflammatory status during an inflammatory response (Dall'Olio et al., 2013). Accordingly, diverse inflammatory diseases were already associated with specific alterations in the protein glycosylation profile (Gornik and Lauc, 2008). Furthermore, physiological aging and age-related diseases, namely dementia and the progeroid disease Cockayne syndrome (Vanhooren et al., 2010), have also been associated with specific changes in the *N*-glycan profile, in particular an increase in an agalactosylated core-α-1,6-fucosylated biantennary *N*-glycan (NG0A2F) and a decrease in a bigalactosylated, core-α-1,6-fucosylted biantennary *N*-glycan (NA2F). These findings lead to the development of the GlycoAge Test (=log[NG0A2F/NA2F]) as an estimate for the biological age of an individual (Vanhooren et al., 2010). The same alterations have recently also been reported for individuals suffering from post-traumatic stress disorder (PTSD), indicating a premature aging phenotype associated with traumatic stress exposure (Moreno-Villanueva et al., 2013).

Building on this knowledge, we hypothesized that the serum *N*-glycan profile would significantly differ in depressed individuals compared to age-matched controls. Furthermore, we hypothesized that alterations in the *N*-glycosylation pattern were related to an increased inflammatory status, and therefore tested for an association with the serum levels of IL-6, TNF-α, and CRP. Given the impact of adverse early life experiences on MDD disease outcomes (e.g., Nelson et al., 2017), we additionally run exploratory analyses to assess the influence of adverse early life experiences (in particular childhood sexual abuse) on the biological changes associated with MDD.

2. Materials and methods

2.1. Study participants

In total, 44 individuals – 22 women (age range: 50–69 years) with Major Depressive Disorder (MDD group) and 22 age-matched control subjects – participated in the study. All study participants provided written informed consent. The study was approved by the Ethics Committee of the Hanover Medical School and was conducted in line with the Declaration of Helsinki (World Medical Association, 2013). To account for the known gender-specific differences in the serum *N*-glycan profile (Ding et al., 2011), we recruited only female MDD patients and controls. The MDD group consisted of individuals with a diagnosis of Major Depressive Disorder according to DSM-IV (American Psychiatric Association, 2000) who received an inpatient treatment at the AMEOS Clinic in Hildesheim, Germany. Acute depressive symptom severity was assessed by the Beck Depression Inventory II (BDI-II, self-report) (Hautzinger et al., 2006). Controls were recruited via public advertisement by posters in public institutions (e.g., supermarkets, gyms). Prior to study participation, the study personnel asked the control subjects whether they had experienced any depressive disorders in their lifetime as well as about a history of depression in two generations before (parents, grandparents). The healthy control group also completed the BDI-II to exclude any individuals with acute symptoms of depression. Study participants further completed the Essener Trauma Inventory (ETI, self-report) (Tagay et al., 2007) for diagnosis of comorbid PTSD. Data on the ETI was missing for two MDD patients. Based on the ETI, the subjects were categorized as positive for a history of childhood sexual abuse if they reported to have personally experienced one of the following two items: 1) "Sexual abuse by a family member during childhood or adolescence", 2) "Sexual abuse by a stranger during childhood or adolescence". Furthermore, we assessed covariates with known influences on inflammation, such as smoking, physical activity (both measured by a dichotomous item with the answer categories "yes" and "no" in self-report), and the body mass index (BMI). Exclusion criteria for both groups were any history of severe head trauma, neuro-psychiatric diseases including Parkinson's disease, Alzheimer's disease, and schizophrenia and any other clinically relevant neurologic or psychiatric disorders. Moreover, subjects with anemia, severe immune alterations, autoimmune diseases, and cancer, as well as subjects reporting the current intake of medication with known effects on the immune system (e.g., immunosuppressors, cytostatic agents, recent vaccines) were excluded from the study. Additionally, signs for a clinically relevant acute inflammatory response led to the exclusion from this study, which was the case in one MDD patient (IL6: 13.0 ng/l, CRP: 36.3 mg/l, TNF-α: 8.6 ng/l) and one control subject (IL6: 17 ng/l, CRP: 41.7 mg/l, TNF-α: 12.0 ng/l). Therefore, the final study cohort for all statistical analyses consisted of $N = 21$ MDD patients and $N = 21$ control subjects.

2.2. Analysis of the serum N-glycosylation profile

Whole blood was collected by venous puncture into serum collection tubes (Sarstedt, Nümbrecht, Germany) between 7 a.m. and 2 p.m. Precise data on daytime of blood collection was available for $N = 35$ study participants. A group comparison showed no difference in mean time of blood collection between MDD patients and control subjects (Table 1). Immediately after blood sampling, whole blood samples were centrifuged at 3000*g* for 10 min at 4 °C for serum collection. Serum aliquots of 250 µl were immediately frozen and stored at − 80 °C until batch-wise analysis. Frozen serum samples were shipped on dry ice to the VIB Center for Inflammation Research (Ghent, Belgium). Using an ultra-sensitive technique for the quantification and sequencing of *N*-linked glycans by "DNA Sequencer Adapted-Fluorophore Assisted Carbohydrate Electrophoresis" (DSA-FACE), the *N*-glycosylation profile of circulating serum proteins was analyzed in batches as described by Vanhooren et al. (2008). In short, following denaturation of the serum proteins, *N*-glycans were separated from the proteins through enzymatic digestion. The free *N*-glycans were then fluorescently labeled, desialylated, and separated on a DNA sequencer (3130 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA). The nine most prominent peaks in the human serum *N*-glycan profile (Liu et al., 2007) were quantified and the peak sizes, which correspond to the relative concentration of the respective oligosaccharide structures, were normalized to total signal intensity. For analyses, we calculated the GlycoAge Test as the log ratio of peak 1 to peak 6 [log10(peak1/peak6)]