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Interplay between innate immunity and iron metabolism after acute pancreatitis

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ABSTRACT

Emerging evidence shows that chronic low-grade inflammation and changes in markers of innate immunity are implicated in a range of metabolic abnormalities following an episode of acute pancreatitis. Also, deranged iron metabolism has been linked to type 2 diabetes mellitus, gestational diabetes, and new-onset diabetes after pancreatitis – the conditions characterized by high haemoglobin glycation index (HGI). This study aimed to investigate the associations between markers of innate immunity and iron metabolism in individuals after acute pancreatitis. Fasting blood samples were collected to analyse lipopolysaccharide binding protein (LBP), interleukin (IL)-6, tumor necrosis factor-α, hepcidin, ferritin, soluble transferrin receptor, HbA1c, and glucose. Participants were categorized into two groups: low HGI and high HGI. Linear regression analyses were conducted, and potential confounders (age, sex, ethnicity, body mass index, diabetes mellitus status, smoking status, aetiology of pancreatitis, duration, recurrence, and severity of pancreatitis) were adjusted for in 5 statistical models. A total of 93 patients following an episode of acute pancreatitis were included, of who 40 (43%) had high HGI. In the overall cohort, LBP was significantly associated with hepcidin and ferritin, and IL-6 was significantly associated with hepcidin, consistently in all the models. Further, LBP contributed to 7.7% and 9.5% of variance in hepcidin and ferritin levels, respectively, whereas IL-6 contributed to 5.3% of hepcidin variance. Upon subgroup analysis, the observed LBP associations were maintained in the high HGI subgroup only and the IL-6 association in the low HGI subgroup only. No consistently significant associations were found between any of the other markers. The interplay between LBP, IL-6, hepcidin, and ferritin characterizes metabolic derangements after acute pancreatitis and may play a role in the pathogenesis of new-onset diabetes after pancreatitis.

1. Introduction

Acute pancreatitis (AP)[1] is one of the most common gastrointestinal diseases [1], with an incidence of 34 cases per 100,000 person-years and accounting for nearly two deaths per 100,000 person-years [2]. Originally thought to be a completely reversible disease, emerging studies indicate that chronic low-grade inflammation persists after hospital discharge in AP patients, and is linked to a range of metabolic abnormalities including insulin resistance, diabetes mellitus (DM), and obesity [3]. Almost 40% of patients with at least one episode of AP develop new-onset pre-diabetes or diabetes after pancreatitis (NODAP)[2] [4]. Furthermore, patients with at least one episode of AP are at a 2.5 times increased risk of developing NODAP, compared to the general population [5,6], and NODAP is the largest contributor to diabetes of the exocrine pancreas [7]. Despite these epidemiological data, the mechanisms underlying metabolic abnormalities after AP have only recently started being investigated [4,8–20].

Accumulating evidence shows that chronic low-grade inflammation is a common link between AP-related metabolic abnormalities, with the immune system being a central pathogenetic factor in the development of these derangements [9,11,18]. Research suggests that gut-derived lipopolysaccharide of gram-negative bacteria may, at least in part, cause the manifestation of chronic low-grade inflammation in a phenomenon referred to as 'metabolic endotoxemia' [19,21]. Lipopolysaccharide binding protein (LBP),[3] a glycoprotein involved in the afferent arm of innate immunity [22], adheres to lipopolysaccharide enhancing its immunostimulatory capacity. A subsequent inflammatory response results in increased expression of pro-inflammatory cytokines

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[1] Acute pancreatitis.
[2] New-onset pre-diabetes or diabetes after pancreatitis.
[3] Lipopolysaccharide binding protein.

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such as interleukin-6 (IL-6)[4] and tumor necrosis factor-α (TNF-α)[5] [19,23], which are involved in the efferent arm of innate immunity [22]. In addition, iron is increasingly being implicated in range of metabolic diseases including prediabetes [24,25], DM [26,27,28], metabolic syndrome [29,30], obesity [31–33], and NODAP [34]. Alongside the innate immune system, iron may be a potential driving force underlying the chronic low-grade inflammation [35–37]. Iron is a potent pro-oxidant and, therefore, promotes oxidative stress through the release of reactive oxygen species and pro-inflammatory cytokines [38]. A resulting pro-inflammatory milieu may contribute to pancreatic β-cell damage [35,37], insulin resistance in adipose [39,40] and skeletal tissues [41], and hepatic dysfunction [38]. Given the role of innate immunity and markers of iron metabolism (the most commonly studied of which are hepcidin, ferritin, and soluble transferrin receptor (sTfR)) in chronic low-grade inflammation, we hypothesised that the crosstalk between innate immune system and iron metabolism plays a role in the pathogenesis of deranged glucose metabolism following an episode of AP.

The aim of this study was to investigate the associations between markers of innate immunity and iron metabolism in individuals after AP overall, as well as in subgroups stratified by haemoglobin glycation index (HGI).

2. Materials and methods

2.1. Study design

This was a cross-sectional study of patients after an episode of AP, approved by the Health and Disability Ethics Committee (13/STH/182).

2.2. Study population

Individuals were eligible for the study if they (1) had a prospectively established diagnosis of AP in line with the most recent international guidelines [42]; (2) were at least 18 years of age; (3) resided in Auckland at the time of the study; and (4) provided informed consent.

Individuals were not eligible for the study if they (1) had a present or prior diagnosis of chronic pancreatitis; (2) had post-endoscopic retrograde cholangiopancreatography pancreatitis; (3) had an intraoperative diagnosis of pancreatitis; (4) were pregnant at the time of the study; or (5) had malignancy.

2.3. Laboratory measurements

Participants were required to fast for a minimum of eight hours before blood collection. Venous blood was collected for ferritin, fasting blood glucose (FBG), glycated haemoglobin (HbA1c), IL-6, TNF-α, LBP, hepcidin, and sTfR by a certified phlebotomist at LabPlus – an International Accreditation New Zealand accredited medical laboratory (Auckland City Hospital).

Fasting blood glucose was measured using an enzymatic colorimetric assay (F. Hoffmann-La Roche). Glycated haemoglobin A1c was measured using the boronate affinity chromatography assay (© 2015 Roche Products and Roche Diagnostics NZ). Ferritin was measured using the electrochemiluminescence immunoassay (© Roche Products and Roche Diagnostics NZ).

Interleukin-6 and TNF-α were measured using the MILLIPLEX® MAP Human metabolic hormone magnetic bead panel based on the Luminex xMAP® (Luminex Corporation, Austin, Texas, USA, 1995) technology. Results were quantified (ng/mL) based on fluorescent reporter signals recorded by the Luminex xPONENT® software (MILLIPLEX® Analyst 5.1).

Lipopolysaccharide binding protein, hepcidin, and sTfR were measured using commercially available enzyme-linked immunosorbent assays, according to the manufacturers' instructions. The coefficient of variation was < 10% for LBP. The intra-assay and inter-assay variation for hepcidin was < 10% and < 15%, respectively; and for sTfR was < 5% and < 10%, respectively. The Rayto Microplate Reader (V-2100C, Santa Fe, Granada, Spain), with an absorbance range of 405–630 nm was used.

2.4. Definitions

Aetiology was categorized as biliary, alcohol-induced, or other.

Body Mass Index (BMI) (kg/m^2) was defined as the participant's weight (kg) over height (m) squared, and determined using a digital medical scale and stadiometer (Health o meter Professional, 2013, © Pelstar, LLC, IL, USA). Participants were asked to remove shoes and head attire for height measurement, and to remove shoes, jackets, belts, accessories, and empty pocket contents for weight measurement. Participants were then categorized into four groups: underweight (< 18.5 kg/m^2), healthy (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese (≥ 30 kg/m^2) according to the most recent World Health Organisation guidelines [43].

Diabetes Mellitus was defined as FBG ≥ 7.0 mmol/L and/or HbA1c ≥ 48 mmol/mol at the time of the study, in line with recommendations of the American Diabetes Association [44,45].

Duration was categorized into < 12 months, 12–36 months, and > 36 months based on the time from the first hospital admission attributable to AP to the time of the study.

Haemoglobin Glycation Index was calculated as observed HbA1c – estimated HbA1c [46]. The study cohort was divided into low (HGI < 0) or high HGI (HGI ≥ 0) subgroups. Emerging as a robust measure of blood glucose control, HGI addresses the propensity for some individuals to have consistently lower or higher observed HbA1c than expected, due to biological variation, even in those with comparable plasma glucose concentrations [46–50].

Recurrence of AP was defined as readmission to the hospital with confirmed diagnosis of AP since the time from the first hospital admission attributable to AP to the time of the study. Recurrence was categorized into three groups: no recurrence (0), 1–2 episodes, and > 2 episodes.

Severity of AP was determined as mild, moderate or severe/critical based on the 2012 Determinant-based classification [51].

Smoking status was recorded as a binary outcome, i.e. 'no' or 'yes' based on participants response to whether they currently smoke cigarettes or tobacco products daily.

2.5. Statistical analyses

All statistical analyses were performed using SPSS 24 for Windows (IBM Corp., 2015). Discrete patient characteristics were displayed as count frequencies, while continuous patient characteristics were presented as median and interquartile ranges (IQR). The subsequent statistical analysis was conducted in three steps.

First, linear regression analysis was conducted to investigate the associations between markers of innate immunity (LBP, IL-6, and TNF-α), and markers of iron metabolism (hepcidin, ferritin, and sTfR) in the overall cohort. The analysis was conducted using five models. Model 1 was an unadjusted model; model 2 was adjusted for age, sex, and ethnicity; model 3 was adjusted for age, sex, ethnicity, BMI, smoking status, and DM status; model 4 was adjusted for age, sex, ethnicity, BMI, smoking status, DM status, aetiology, duration, recurrence, and severity of AP; and model 5 was adjusted for those covariates found to be statistically significant in model 4. All data were presented as β-coefficients with corresponding 95% confidence intervals (CI) and *p*-value.

Second, linear regression analysis was conducted to derive an

[4] Interleukin-6.
[5] Tumor necrosis factor-α.