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COVID-19: NAD^+ deficiency may predispose the aged, obese and type2 diabetics to mortality through its effect on SIRT1 activity

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A B S T R A C T

The SARS-CoV-2 hyperinflammatory response is associated with high mortality. This hypothesis suggests that a deficiency of nicotinamide adenine dinucleotide (NAD^+) may be the primary factor related to the SARS-Cov-2 disease spectrum and the risk for mortality, as subclinical nutritional deficiencies may be unmasked by any significant increase in oxidative stress.

NAD^+ levels decline with age and are also reduced in conditions associated with oxidative stress as occurs with hypertension, diabetes and obesity. These groups have also been observed to have high mortality following infection with COVID-19. Further consumption of NAD^+ in a pre-existent depleted state is more likely to cause progression to the hyperinflammatory stage of the disease through its limiting effects on the production of SIRT1.

This provides a unifying hypothesis as to why these groups are at high risk of mortality and suggests that nutritional support with NAD^+ and SIRT1 activators, could minimise disease severity if administered prophylactically and or therapeutically. The significance of this, if proven, has far-reaching consequences in the management of COVID-19 especially in third world countries, where resources and finances are limited.

Background

COVID-19 may be asymptomatic or manifest in 3 clinical phases, an initial upper respiratory tract infection, with a few patients thereafter progressing to a pneumonic phase, and an even smaller number to the hyperinflammatory phase which may be lethal [1]. The aim of any therapy would be to intervene at an early stage, either prophylactically or therapeutically, to prevent progression of the disease to a point where mechanical ventilation (MV) is required, or significant organ dysfunction occurs [2].

Risk factors for a poor outcome include older age, comorbidity (in particular diabetes, hypertension and cardiac disease), non-asthmatic respiratory disease, obesity, immunosuppression and male sex [2,3]. The independent associations of advancing age, male sex, chronic respiratory conditions (though not well controlled asthma), chronic cardiac and chronic neurological disease with in-hospital mortality, are in line with other international reports [4]. It is difficult however to determine why these conditions specifically are linked to mortality.

Docherty et al. report that severe SARS-CoV-2 infections are rare in those under 18 years of age, comprising only 1.4% of those admitted to hospital. Only 0.8% of those in this study were under 5 years of age, and this "J" shaped age distribution was starkly different from the "U" shaped distribution seen in seasonal influenza [5]. It has not been clear from observational studies however, why SARS-CoV-2 mostly spared children, but it has been speculated that it is due to differential expression of the ACE2 receptor in the developing lung [6]. Similarly, pregnancy has not been reported to be associated with mortality, in contrast to the situation with influenza [4,7].

While the general concept of an excessive or uncontrolled release of pro-inflammatory cytokines is well known, an actual definition of what a hyperinflammatory response or "Cytokine Storm" is, is lacking. Furthermore, there is a poor understanding of the molecular events that precipitate this response and the contribution it makes to pathogenesis. It is also not known what therapeutic strategies might be used to prevent this catastrophic progression of the disease or lessen its severity once initiated [8].

In this phase, there is an unbalanced and exacerbated inflammatory response with the release of inter alia, tumor necrosis factor (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6), as pro-inflammatory mediators together with interleukin 10 (IL-10) and interferon β as anti-inflammatory mediators. The complex interactions between TNF-α, the interleukins, chemokines and interferons in SARS-CoV-2 are currently poorly understood; however, they are associated with and related to a significant viraemia [9,10].

The fact that most international studies have indicated that certain risk factors were common suggested that a similar systemic abnormality

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might be present in those at high risk, predisposing to severe illness or mortality.

In this context, the nicotinamide adenine dinucleotide (NAD^+)- and zinc-dependent molecule, the Silent Information Regulator 1 (SIRT1) represents a potential common thread in the aetiology of the hyperinflammatory response and increased mortality.

SARS-CoV-2 binding

SARS-CoV-2 targets and binds to the angiotensin-converting enzyme 2 receptor (ACE2R), a membrane-associated aminopeptidase also expressed in the vascular endothelium, renal and cardiovascular tissue, and small intestine, testis, and respiratory epithelia [11]. The ACE2R acts as the host cell receptor for the virus which binds via the spike protein on the viral capsid [12]. This stimulates clathrin-dependent endocytosis of both the ACE2R and virus, events that are essential for infectivity. This process induces ADAM 17 activity which reduces expression of ACE2 on the cell surface [13].

Nicotinamide adenine dinucleotide (NAD^+)

NAD^+ is a cofactor found in every cell of the body, and it is involved in multiple metabolic pathways. It is a fundamental housekeeping molecule that catalyses electron transfer in metabolic reduction–oxidation reactions, functioning as an electron shuttle in the production of adenosine triphosphate (ATP).

Increased age is a strong predictor of SARS-CoV-2-associated in-hospital mortality after adjusting for comorbidity [6]. Older patients have also been identified as having the lowest levels of NAD^+ [14], while, conversely, those with the lowest risk, infants and children have the highest levels.

The decline in NAD^+ levels with ageing is mainly dependent on CD38, a 45 kDa transmembrane molecule, encoded on chromosome 4. In leukocytes, it acts as a receptor in adhesion and signalling pathways [15]. CD38 expression is increased with insulin resistance, and may exacerbate the age-dependent decline of NAD^+ [9]. NAD^+ and NADP profoundly affect age-influencing factors such as oxidative stress and mitochondrial activity, and NAD^+-dependent sirtuins also mediate the ageing process [10]. As humans age, antioxidant defence mechanisms such as glutathione production are also depleted and the associated increase in reactive oxidative species (ROS) causes all cells [16] to enter a state of pseudohypoxia in which the ratio of NAD^+/NADP declines [17–19].

Oxidative stress also activates the NAD^+-dependent enzyme, poly ADP ribose polymerase 1 (PARP1) [20]. Hyperactivity of PARP1 results in depletion of cellular NAD^+ pools, leading to ATP deficiency, energy loss, and subsequent cell death. These processes have the potential to enhance the pro-inflammatory cascade.

NAD^+ deficiency impairs SIRT1 function [21] and its successful activation. Whereas extreme niacin deficiency is associated with the development of pellagra, more subtle decreases occur in diabetes, ageing and hypertension with resultant attenuation of responsiveness to inflammatory stimuli [22].

Silent Information Regulator 1 (SIRT1)

Sirtuins are an ancient family of seven NAD^+-dependent deacylase and mono-ADP-ribosyl transferase signalling proteins that are intrinsically involved in metabolic regulation and cellular homeostasis. Of particular interest is SIRT1, which downregulates ADAM 17 (A Disintegrin and Metalloproteinase Domain 17), also called TNF-α converting enzyme (TACE), by increasing expression of TIMP3 the gene that encodes for tissue metalloproteinase inhibitor 3 [23]. In so doing it decreases levels of TNF-α, IL-1b and IL-6. An increase in TNF-α causes SIRT1 to down-regulate ADAM 17, thereby controlling TNF-α formation in a negative feedback loop that secondarily influences IL-1b and IL-6 production, which are dependent on TNF-α [23].

SIRT1 is known to play a crucial role in obesity-associated metabolic diseases, cancer, ageing, cellular senescence, cardiac ageing and stress, prion-mediated neurodegeneration, inflammatory signalling in response to environmental stress, embryonal development of the heart, brain, spinal cord and dorsal root ganglia, and placental cell survival [24]. In its inactive or open state, it contains a Zn^{++} module and an NAD^+ -binding site [25].

Whereas certain conditions such as ulcerative colitis, Crohn's disease, short bowel syndrome, renal failure, alcoholism, and inadequate meat intake are specifically associated with zinc deficiency there is evidence that zinc intake among older adults might be marginal. An analysis of the Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994 data found that 35–45% of adults aged 60 years or older had a zinc intake below estimated average requirements [26].

When NAD^+ binds to the SIRT1 molecule in the presence of the Zn^{++} -binding module, it undergoes a structural change, enveloping the NAD^+ molecule and causing it to be “closed” or activated [25]. The presence of both the Zn^{++} and the NAD^+ moieties are imperative for its function.

SIRT1 downregulates ADAM17 and cytokine production

ADAM17 is a proteinase encoding gene. TNF-α and the cytokine receptor for IL-6 must be proteolytically cleaved in order to be systemically active, and ADAM17 provides this function. If ADAM17 expression is not downregulated by SIRT1, TNF-α and IL-6 are released, resulting in an uncontrolled hyperinflammatory response as may occur with COVID-19 [23,27–30]. SIRT 1, by inhibition of ADAM17 and thereby TNF-α and IL-6, performs an anti-inflammatory function [31–38].

If oxidative stress is severe, increased ADAM17 attempts to ameliorate tissue injury by converting active iron ($Fe2^+$) to its inert form ($Fe3^+$) which is stored in hepatocytes and macrophages and as ferritin by means of the Fenton reaction ($Fe2^+ + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^-$), ($Fe^{3+} + H^2O^2 \rightarrow Fe^{2+} + HO_2^{\cdot} + H^+$). This also potentially transforms haemoglobin to methaemoglobin, reducing its capacity to bind to oxygen [39].

COVID-19 replication and SIRT1

SIRT1 not only controls and modifies the inflammatory response, but along with the Sirtuin family (SIRT1–7) is also a primary defence against DNA and RNA viral pathogens [40]. In some respiratory infections and cardiovascular conditions, SIRT1 promotes autophagy (the destruction of damaged or redundant cellular components occurring in vacuoles within the cell), and in so doing inhibits apoptosis and provides protection against hypoxic stress [37–40].

Upregulation of SIRT1 directly decreases viral replication and inhibits the activation of ADAM17, thereby decreasing TNF-α, IL-1b and IL-6. Conversely depletion of SIRT1 allows for increased viral replication with little or no inhibition of ADAM17 activity, causing uncontrolled increases in TNF-α, IL-6 and IL-1b. Whereas an increase in TNF-α would usually increase SIRT1 activity to downregulate ADAM17, in the presence of a deficiency of NAD^+ or Zn^{++}, this would not occur due to insufficient activation of SIRT1, causing an unchecked increase in TNF-α.

In both obesity and type 2 diabetes mellitus, intracellular NAD^+ levels are decreased in multiple tissues, including adipose tissue, skeletal muscle, liver and the hypothalamus. [41] Furthermore, both conditions are characterised by low-grade inflammation associated with activation of both IL6 and TNF-α [42,43]. Obesity or type 2 diabetes mellitus would increase the risk for cytokine storm due to an inability to activate SIRT1.

SIRT1 maintains vascular endothelial function, preventing or

reducing the potential for the metabolic syndrome, ischaemia–reperfusion injury and inflammation in obesity. With increasing age however, NAD^+ levels and sirtuin activity decline and this is exacerbated by obesity and sedentary lifestyles [22]. SIRT1 is an effective inhibitor of oxidative stress in vascular endothelial cells (EC) [44] via various signalling pathways [45].

The endothelial glycocalyx (EG) is a web of membrane-bound glycoproteins on the luminal side of endothelial cells, associated with various glycosaminoglycans that cover the vascular endothelium [46]. The EG separates cellular blood components from the endothelium and maintains osmotic tension of the intravascular compartment [44,45].

Conditions causing damage to, and shedding or fragmentation of the EG, (as seen in SARSCoV-2 under severe oxidative stress induced by the hyperinflammatory response), exposes the endothelium, allowing adhesion, clumping and activation of platelets with degranulation and release of vasoactive substances. The EG has anticoagulant properties as it is a binding site for mediators such as heparin cofactor II, antithrombin, thrombomodulin and tissue factor pathway inhibitor (TFPI). Heparin cofactor II and dermatan sulphate inhibit thrombin, and antithrombin activity is enhanced when bound to heparan sulphate. Conversely, exposure of the endothelial cell surface protein, thrombomodulin, which contains a cofactor for thrombin, chondroitin sulphate, promotes coagulation via activation of tissue factor [46] as seen in SARCoV-2.

The EG is already compromised in systemic inflammatory states, such as diabetes, hyperglycaemia, surgery, trauma and sepsis [46]. Under conditions of more severe oxidative stress, as in the hyperinflammatory response, widespread damage may lead to its destruction, with the occurrence of capillary leak and oedema formation, accelerated inflammation, platelet aggregation, hypercoaguability and a loss of vascular responsiveness [47]. Inflammatory mediators that are implicated in this process are TNF-α, bradykinin, C-reactive protein and mast cell tryptase.

Given the above, it is possible that activation of SIRT1 may be a crucial factor in the prevention of the hyperinflammatory response and may be necessary for a successful defence against viral attack. Vulnerable patient groups would potentially be less likely or unable to ensure sufficient activation of SIRT1 due to low NAD^+ levels or associated nutritional deficiencies including Zn^{++}, and as such contribute to an inability to control viral replication and reduce the uncontrolled expression of pro-inflammatory cytokines.

Conclusion

The SARS-CoV-2 hyperinflammatory response is associated with high mortality. A deficiency of NAD^+, in the context of an elevated CD38, may be the primary factor related to the SARS-Cov-2 disease spectrum and the risk of mortality, as subclinical nutritional deficiencies may be unmasked by any significant increase in oxidative stress.

NAD^+ levels decline with age and are also reduced in conditions associated with oxidative stress as occurs with hypertension, diabetes and obesity. These same groups have also been observed to have high mortality following infection with COVID-19. Further consumption of NAD^+ in a pre-existent depleted state is more likely to cause progression to the hyperinflammatory stage of the disease through its limiting effects on the production of SIRT1.

Given that activation of SIRT1 is dependent on the availability of NAD^+ and zinc and that high levels of oxidative stress deplete NAD^+, thereby decreasing SIRT1 activity, nutritional support with NAD^+ precursors and SIRT1 activators, could minimise disease severity if administered prophylactically and or therapeutically. The significance of this hypothesis, if proven, has far-reaching consequences in the management of COVID-19 especially in third world countries, where resources and finances are limited.

Grants and support

NA.

Hypothesis

We hypothesize that reduced Nicotinamide Adenine Dinucleotide (NAD^+) levels with consequent deficient activity of the NAD^+ dependent molecule SIRT1, which modulates cytokine production, may be the factor that predisposes the aged, obese, type 2 diabetics and other vulnerable groups to an increased mortality.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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