Rheumatoid Arthritis and Stroke: is Homocysteine a Linking Factor?

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Abstract: Ever since increase in homocyseteine (hcy) level termed as hyperhomocysteinemia (hhcy) has been recognized as one of causal factors for an independent factor for the atherothromobosis and endovascular injury, it has become an element of great interest of research. Homocysteine (hcy) has close association (between hcy and different diseases) particularly in rheumatoid arthritis (RA) and in cardio and cerebro-vascular diseases. The capability of hcy to the vascular damage and structurally modifying specific proteins, resulting in formation of neo-antigens may also be a triggering factor of autoimmune reactions and thus hcy has probability to present itself as an initiating factor in autoimmune disease like RA. These all circumstances point towards potentially relevant role of hcy in the onset of specific autoimmune disease. Hcy causes or plays a role in the progression of the associated cardiovascular and cerebrovascular damage through its inflammatory property. Immuno-inflammatory activation may contribute to the increase in hcy level which in turn may add up to the injury of the specific organ in specific diseases (like vessel injury in cardio and cerebrovascular disease, synovial tissue in RA). This bi-directional link appears to connect hcy and the auto-immune disease (immuno-inflammatory activation). In this review we have tried to present the potential relation of hcy as a common linking factor between stroke and rheumatic arthritis, through its bi-directional property. Through this current opinion we have tried to put forth our opinion that hcy is one of the common causal factors in stroke well as in rheumatoid arthritis. This carries an interesting clinical importance in control and prevention of these two diseases of different entity at a common point.

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Introduction:

Hcy, an intermediate in protein metabolism, is involved in conversion of the amino acid methionine (met) to cysteine (cys) or in remethylation to form methionine. Hcy has already been recognized as a risk factor for atherosclerotic vascular disease and many studies done have found adverse influence of increased hcy on endothelial cells, vascular smooth muscle cells, connective tissue, interactions with plasma lipoproteins, clotting factors and platelets. Different reasons for the increase in hcy level have been sought. Increase in hcy concentrations are often the result of decreased activity of key enzymes involved in metabolic pathways of hcy. A mutation in the methylene tetrahydrofolatereductase (MTHFR) gene leads to mild to moderate hhcy has been found to cause hcy and is associated with premature cerebrovascular disease found in 15 percent of patients.[1,2] Less often, the cause of hhcy is heterozygous cystathionine b-synthase (CBS) deficiency. Hhcy can also be acquired as the result of dietary deficiencies of folate, vitamin B_{12} and/or vitamin B_6. Certain drugs, especially vitamin antagonists such as methotrexate and anticonvulsants, can cause hhcy. Notable hcy elevations can also occur in illnesses such as chronic kidney disease, RA or hypothyroidism.[3]

Since hcy is a common but an important responsible factor in course of stroke and RA, our purpose through this review is to present a clear and concise role of hcy as a common factor responsible for both. We have tried to establish the possible role of hcy as a linking factor between RA and stroke which carries an interesting clinical importance in its control and prevention.

Implication of autoimmunity and inflammatory action in Stroke and RA

Talking about RA, it is a common, chronic inflammatory joint disease. Mostly there is an often seen persisting joint inflammation, despite the treatment with disease modifying antirheumatic drugs (DMARD) but the course of disease may vary. Many existing literatures have reported increased mortality in association with RA.[4-8] RA is also considered an autoimmune disease.[9,10] The destructive progression of the disease is driven by the load from autoimmunity and the overall systemic and articular inflammatory mediators.[11] NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is up

regulated in RA and cytokines such as TNFα (tumor necrosis alpha) that activate NF-κB are elevated in the synovial fluid of patients with RA[12,13] Cytokines IL-6, IL-8 (Interleukins) are also deeply involved in the pathogenesis of rheumatoid arthritis (reference cited elsewhere). Ischemic stroke is also a result of inflammation as found through intensive research at the basic and clinical level. In this, there are two important patho-physiological mechanisms involved during ischemic stroke and are oxidative stress and inflammation. Although there are different mechanisms involved, but growing evidence support mainly inflammation and oxidative stress.[14] Antioxidant defenses is not well developed in the brain, so reactive oxygen species (ROS) and other free radicals/oxidants, released by inflammatory cells, threaten tissue viability in the vicinity of the ischemic core. The most studied cytokines related to inflammation in acute ischemic stroke are TNF-α, the ILs, IL-1β, IL-6, IL-20, IL-10 and transforming growth factor (TGF)-β. While IL-1β and TNF-α, has been dubbed to exacerbate cerebral injury, TGF-β and IL-10 has been implicated to be neuro-protective. [15,16] Elevation in production of pro-inflammatory cytokines and lower levels of the anti-inflammatory IL-10 are related to larger infarctions and poorer clinical outcome. These cytokines playing their respective roles in the disease course point toward the strong possibility of inflammation playing major part in cerebrovascular disease namely stroke.

The bi-directional property of HCY:

Hcy may be a triggering factor of autoimmune responses, with putatively relevant implications for the development of vascular disease and the onset of auto immune-disease. Different studies done by different researchers have demonstrated intriguing effects for hcy. Currently, the fact that Hcy plasma levels are higher not only in RA but also in other autoimmune diseases like in MS patients than in controls, and the observation that such an increase seems unrelated to vitamin deficiency[17] seem to be acknowledged and thus possibility of hcy as being the initiating factor for autoimmunity and inflammatory process and the increase of hcy being the result of autoimmunity (a bidirectional property of hcy) are the points of interest for researches recently.

A) Association of Immuno-inflammatory activation and hcy increase:

The findings for hcy either in the enhancement of inflammatory activation and in the autoimmunity triggering mechanisms has opened a new chapter on a possible role for hcy not only in the development of accelerated atherosclerosis (AA), but also in the pathogenesis of auto immune disease(AD) like RA, or in cerebrovascular disease like stroke. The development of hhcy and the mechanism underlying its development as a consequence of a persistent immuno-inflammatory activation, although not completely clarified, are probably multiple and intriguing. Hcy enhances the production of molecules such as IL-6, IL-8, and monocyte chemo attractant protein-1 by monocyte-macrophages and endothelial cells, and nitric oxide, matrix metalloproteinase-9 and vascular cell adhesion molecule-1 (VCAM-1) by vascular smooth muscle cells. [18-22] Thereby suggesting a possible additional role for hcy in the inflammatory process supporting atherogenesis. Some bio-humoral parameters of inflammation, such as the circulating levels of soluble receptors for different cytokines (IL-2sRα sTNF-R75) [24–26], adhesion molecules (sICAM-1)[26], and C-reactive protein (CRP) [27,28] have been found to have positive relationship with the concentration of hcy. Hcy significantly enhances IL-6 and IL-8 production by synoviocytes from RA patients, particularly in the presence of co-stimulation with IL-1[29]. Synoviocytes are the cells mainly involved in the development of cartilage damage in chronic inflammatory joint diseases, particularly RA.[30] These cells play a relevant pathogenetic role by producing several cytokines, including IL-6 and IL-8, when triggered by IL-1.[31-34] In fact, either IL-6 and IL-8 are critically involved in the development of RA, and levels of both cytokines correlate with the clinical behavior of the disease.[35,36] A solid body of evidence from study done by Georganas C et al demonstrates that NF-kB is constitutively activated in RA synovial tissue.[37] These concepts support the hypothesis that hcy induced cytokine production in synovial cells is mediated via NF-κB pathway activation, and that NF-κB constitutive activation could account for the particular sensitivity to Hcy of RA synoviocytes. The expression of inflammatory factors during the onset of atherosclerosis has been linked with the activation of transcription factor NF-κB.[38–40]

1. Increase in Hcy as a pro-inflammatory and immuno-stimulating molecule: The pro-inflammatory and immune modulating properties of hcy have been confirmed through different studies in vitro on several vascular cell-types extending also the spectrum of the molecules involved. Many authors have been able to demonstrate that hcy was able to induce chemokine (IL-8 and/or MCP-1), and chemokine receptor expression by human vascular cells and monocytes.[41,42] These results corroborated by the results from the data obtained in vivo in subjects with hhcy[43] have suggested the importance of hcy in the enhancement of monocyte chemotaxis into the arterial wall representing one of the key events during atherogenesis. There are several other cytokines and pro-inflammatory molecules with a hcy-dependent stimulatory effect, and they include: IL-1β[44] ,IL-6 [44-46], IL-12[44], IL-18[47] , IL-1 receptor antagonist (IL-1ra) [46], adhesion molecules (P-selectin, E-selectin, ICAM-1)[48]. Hcy seems able to up-regulate

the production of ROS, putatively involving the stimulation of IKK kinase (I κA and I κB kinase) responsible, in its turn in the activation of NF-κB thus acting like proinflammatory factor. [49] To add to this with the evidence an investigation led by Lazzerini PE and his colleagues, found the possibility that hcy could play a direct pro-inflammatory activity also in the joints of RA patients with the evidence that hcy is present in RA synovial fluids at a concentration of about 10 μmol/l, the effect of 10–100 μmol/l Hcy±IL-1β was evaluated on IL-6 and IL-8 production by cultured synoviocytes from RA patients. The results of their study showed that hcy enhanced cytokine production in RA synoviocytes (up to 35%) at a concentration measurable in RA joints in vivo, with a clear-cut activation of NF-κB.[29] Considered as a whole, the above mentioned data has strongly recognized the aptitude of hcy to activate the immune system and enhance the inflammatory process. The cause of hcy in the immune activation because of hhcy can also be because of the structure modifying property of hcy. This was also seen by Gao et al.[50] Through the research he was able to show as hcy can modify several class I HLA antigens, including HLA-B27 through disulfide bonding.(In fact, it is well established that the class I HLA-B27 antigen is strongly associated with the development of reactive arthritis). In another study by Chilvers et al, it was also identified cytotoxic T lymphocytes (CTL) in patients with reactive arthritis (ReA) that are capable of specifically lysing autologous cells that had been treated with Hcy in vitro, thus providing a possible pathogenetic link between hcy, HLA-B27 modification and autoimmune reactivity in such disorders[51] .In this way, a bi-directional link seems to connect hcy and the immuno-inflammatory activation characterizing AD, in which immuno-inflammatory activation may contribute to hcy increase, and hcy, in its turn, may act as a pro-inflammatory and immuno-stimulating molecule putatively cooperating at the injury of the disease-specific target organs, at least in case of RA and in the same way, hcy may be causing the inflammatory process in vessels ultimately causing cerebrovascular disease stroke.

Homocysteine and Rheumatoid Arthritis:

Some clinical features of RA in accumulated disease activity correlates with hhcy.[24] Hhcy also correlates with higher radiological damage.[28] Increase in hcy levels after methionine load correlated with erythrocyte sedimentation rate (ESR) and CRP levels, disability score, degree of pain, and number of painful and swollen joints in 37 RA patients.[52] The effect of high-dose pulsed glucocorticoid treatment on plasma hcy concentration in patients with active RA found a significant 26% hcy reduction, and this effect was both rapid and long-lasting over a 6-month follow-up period. This original finding provided a strong support. Together with the concomitant decrease in CRP observed has provided a further indirect evidence of the link between inflammation and hhcy in RA patients and it also supports that inflammatory state is implicated in the genesis of hhcy. To add the strength of role of hcy in inflammation, a decrease in plasma level of hcy following intensive steroid therapy would be expected together with the overall reduction in inflammation.[53] While some data suggest that hhcy seen in RA is through enhanced and /or accelerated catabolism of vitamins (folate, B12,B6) by the immune activation which is characteristic of RA. The activated immune system may also be playing role in the sulphured amino-acids metabolism[25, 27, 54, 55]

Homocysteine and Stroke:

It all started in 1969 when it was suggested for the first time that there was a connection between increased hcy levels and atherosclerotic diseases[56]. This hypothesis was backed by different successive observational studies [57-59]. A study by other different researchers, concluded that hhcy is an independent risk factor for atherosclerosis of the coronary, cerebral and peripheral blood vessels[57,60, 61].The laboratory studies have also shown that the association is biologically plausible and hhcy produces changes in structure and function of cerebral blood vessels.[62] Although, the common agreement is on hhcy playing an important role in the atherosclerosis, most but not all studies have demonstrated an association between elevated levels of total hcy and stroke.[63-67,71]

Systematic reviews of observational (cohort and case control) studies have consistently shown a strong, positive, and dose-related association between the serum concentration of total homocysteine (tHcy) and the risk of stroke, which is independent of other vascular risk factors.[68, 69] From another different studies every increase of 2.5 μM in plasma hcy can be associated with an increase of stroke risk of about 20 %[68,70] which shows the strength of association of hcy and stroke. Even talking on the molecular level, in the largest meta-analysis to date of studies examining the association between MTHFR and stroke (111 studies), Casas and colleagues found that people who are homozygous (TT) for the MTHFR polymorphism have a significantly greater mean thcy(total plasma hcy) (weighted mean difference 1·93 μmol/L 95% CI 1·38–2·47), and risk of stroke (odds ratio 1·26, 1·14–1·40) than people who are homozygous and unaffected (CC).[72]

Vascular Vulnerability in Rheumatoid Arthritis and HHCY:

A) Cardiovascular Disease and RA

Atherosclerosis is very fast and condition like accelerated atherosclerosis typically found in the course of several autoimmune diseases and they are frequently

associated with cardiovascular damage[73] which was further supported by several studies.[74-77]A cohort study among all residents aged >18 years residing in British Columbia between 1999 and 2003 confirmed that RA is a risk factor for cardiovascular events and shows that the rate ratio for cardiovascular events among subjects with rheumatoid arthritis is highest in young adults and those without known prior cardiovascular events[78]. This relationship between cardiovascular mortality and atherosclerosis severity was pointed out by two studies done separately.[79,80] Cumulative burden of inflammation[76] and the presence of rheumatoid factor (RF) as the reasons responsible in terms of accelerated atherosclerosis for cardiovascular events in RA have been further elucidated by two separate studies by Mardit-Kermers et al and Goodson NJ et al respectively.[81] The results from the Meta-Analysis show that risks of myocardial infarction and stroke are increased in patients with rheumatoid arthritis[82]. A review by Zeyenep Ozbalkan et al clearly established the relationship between the atherosclerosis and cardiovascular mortality through the inflammatory cells such as macrophage, monocyte and T cells playing a common but important role in the development of both RA and atherosclerosis.[83]

Atherothrombotic cardiovascular involvement is particularly frequent and advanced in patients affected with several autoimmune diseases. The cause has been shown to be as increased dyslipidemia, hyperhomocysteinemia, and the medicines usage in RA such as cyclosporine (causing dyslipidemia), methotrxate (causing hhcy), corticosteroids[84] and hhcy, represents a common finding.[85]

B) Cerebrovascular Disease and RA**:**

An increased risk of stroke among patients with RA was demonstrated in 2 studies[75, 78] Inflammation has been related as one of the factors with the cause of vascular injury and ultimately stroke. Atherosclerosis has been recognized as an inflammatory disease[86]that can cause systemic acute phase response.[87,88] TNF may activate endothelial cells, converting them into procoagulant and prothrombotic states. [89-92]TNF is a key cytokine involved in all phases of stroke pathogenesis, including initiation and progression, as well as repair and development of ischemic tolerance

Conclusion:

Hcy is seen increased in inflammatory diseases and autoimmune diseases. Recently, many research articles published have convincingly shown hcy as a factor initiating inflammation and also as a factor initiating autoimmunity, while many literatures have reported the increase of hcy in stroke and other autoimmune diseases mainly in rheumatic arthritis, lupus. Hcy is elevated in stroke. Even the moderate homocysteinemia has been seen responsible in stroke and atherosclerosis. These days, evidence as hcy as a causal factor for stroke is accumulating. Inflammation has also been seen as the causal factor of stroke and autoimmunity is an established cause of RA for which hcy is again one of the important factors. This bidirectional property of hcy carries a special importance. Our conclusion is that elevation of hcy can be one of the initiating factors for stroke and RA. Hcy can be the reason for the initiation of autoimmunity and inflammation and so may be the culprit or the cause of autoimmune disease like RA and inflammatory insults resulting into cerebrovascular accidents like stroke. A particular study with this idea of hcy as a common linking factor for these two diseases has not yet been carried out. Since the risk factor must itself be the cause of disease, not an indirect reflection of a related causal factor, a study on a greater account needs to be done to establish this opinion. Why the vitamin fortification to control hcy is still not effective in controlling RA or stroke? Will someone with elevated hcy alone without other factors resulting in RA or stroke be cured or controlled by the fortification vitamin in intake? These questions remain unanswered even coming out of this heap of articles. That's why a great deal of study with large number of cohort, and case-control study as well as a study of multi-ethnic cohorts is needed to answer these questions and also to answer that remain unanswered up to now. Study at basic molecular level also needs to be done to evaluate the theory of stroke as a causal factor for RA and back up the idea of hcy acting as a common linking factor for both stroke and RA.

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