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ASPIRIN RESISTANCE & LOW OMEGA-3 INDEX – GREATER CHANCES OF CVD MORTALITY ESPECIALLY IN COVID-19

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Abstract: **Low mortality rate of about 1.5% and optimism bias have changed the attitude of general public towards COVID-19. Though COVID-19 cases are increasing in India but mostly they remain in mild stage with 92.6% recovery and 1.48% mortality rate till 10th Nov. 2020 as per WHO reports. According to another study more than 75% of patients experience mild to moderate symptoms and recover without hospitalization. Just 5% patients progress to critical stage with 2-2.5% mortality rate. COVID-19 associated coagulopathy (CAC), the main cause of mortality have been reported mainly in subgroup of patients with comorbidities like CVD, hypertension, diabetes and cancer etc.**

OBJECTIVE:- **Our objective is to identify the factors responsible for COVID-19 symptoms progressing to life threatening CAC and their management.**

DISCUSSION:- **Excessive coagulation with fibrinolytic shutdown is the hallmark of CAC. Cytokine storm induced over expression of Tissue Factor is the potential trigger for CAC. Pharmacological properties of aspirin, like anti-inflammatory and antithrombotic can be used as an evidence to hypothesize its use as a primary preventive treatment for CAC. Most of the patients are already taking aspirin for this purpose. But rising trend in aspirin resistance has made aspirin totally undependable. Omega-3 index (Omega-6/ omega-3 ratio) determines cardiovascular health. More than 8% is cardioprotective and less than 4% leads to major adverse cardiac events. Omega-3 reduces platelet aggregation, coagulation and thrombosis. High omega-6 levels cause coagulation. Omega-6 fatty acid (AA) is the substrate for the synthesis of a variety of proinflammatory/ proaggregatory molecule such as prostaglandin E_2, thromboxane A_2 (TXA_2), & leukotriene B_4.**

CONCLUSION:- **Low Omega-3 index (High Omega-6/ omega-3 ratio) promotes coagulopathy. This can be corrected by adequate omega-3 nutrients (EPA and DHA) supplementation. Administration of EPA & DHA helps in replacing omega 6 fatty acids (such as AA) resulting in reduction of TXA_2. Co-administration of Aspirin, EPA & DHA have shown positive results as an antiplatelet treatment. This also makes aspirin more effective by increasing its sensitivity. We hypothesize that co-adminisration of EPA + DHA + Aspirin may prove to be very useful as a primary preventive antithrombotic option in CVD patients. This can also check progression from mild COVID-19 symptoms to life threatening CAC.**

Keywords: **COVID-19, Aspirin, Omega-3 Fatty Acids, Coagulopathy.**

1. INTRODUCTION

COVID-19 is spreading like wildfire in jungles. All health agencies are trying to enforce strict laws to ensure its containment from spreading by wearing masks, hand sanitization & maintaining social distance. But still thousands of new cases are being reported everyday in India. This clearly shows that those people who are fed up with lockdown & suffer from 'optimism bias' are venturing to go out & that too without wearing masks & with no provision of frequent hand wash & sanitization. In busy markets, there is hardly any room to maintain social distancing. In spite of all this, good number of patients (92.6%) suffering from COVID- 19 experience only mild to moderate symptoms & recover without hospitalization with 1.48% mortality rate. In this largest study of over 44,000 patients with COVID-19, > 75% of cases were mild, 14% were severe, & 5% were critical, with an overall case fatality rate of 2–2.5% (1). Mortality has been reported mainly in subgroup of patients with co-morbidities. Patients without any co-morbidity had a mortality rate of 0.9% whereas those with co-morbidities like cardiovascular disease had mortality rate of 13.2%, Diabetes 9.2%, chronic respiratory disease 8%, hypertension 8.4% and cancer 7.6% (2).Viral infection associated inflammation clearly predisposes patients to prothrombotic states (3). Studies confirm that venous thromboembolism & arterial thrombosis have a high prevalence rate & a detrimental impact on prognosis of hospitalized patients (4, 5). According to various studies, since, aspirin resistance and low omega- 3 index also induce coagulopathy. So, we hypothesize that it might aggravate mild COVID-19 symptoms in CVD patients taking aspirin with low omega-3 index leading to life threatening COVID-19 associated coagulopathy (CAC) resulting in high mortality.

2. PATHOPHYSIOLOGY

2.1 LUNG CENTRIC INITIATION OF COVID-19

Type- 1 & type- 2 pneumocytes have different functions. Type-1 is engaged in gaseous exchange (6, 7) & type-2 maintains elasticity in alveoli for expanding during inhalation & exhalation (6, 8 and 9). The main target of SARS-CoV2 is type-2 pneumocytes because ACE-2 receptors are found in abundance in them as compared to type-1. In type-2 pneumocytes SARS CoV2 infects & enters the cells. There it initiates its RNA multiplication, which gives rise to thousands of virus copies leading to rupturing of pneumocytes & their release (6, 10). This can infect other type-2 pneumocytes. They also enter blood circulation to reach all organs having ACE-2 receptors like cardiovascular system, G.I. tract, kidney etc. (6, 11).

2.2 CYTOKINE STORM

Inflammation is body's first line of defense against infection and injury. After its initiation it should be subsided otherwise it becomes harmful to the body. Body has its own anti-inflammatory system as well to resolve inflammation. Thrombosis & inflammation go hand in hand in COVID-19. They become injurious to the host in direct proportion to the disease severity if it remains unchecked (12). Ruptured type-2 pneumocytes attract macrophages through production of host defense mechanisms which include proinflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNFα), & complement system proteins, all of which specifically raised IL-6 can induce coagulopathy (13). This can lead to endothelial dysfunction, vascular damage, sepsis, hypercoagulation & multi organ injury i.e. Brain, Heart, Lungs, Kidneys and G.I. Tract (Fig 1).

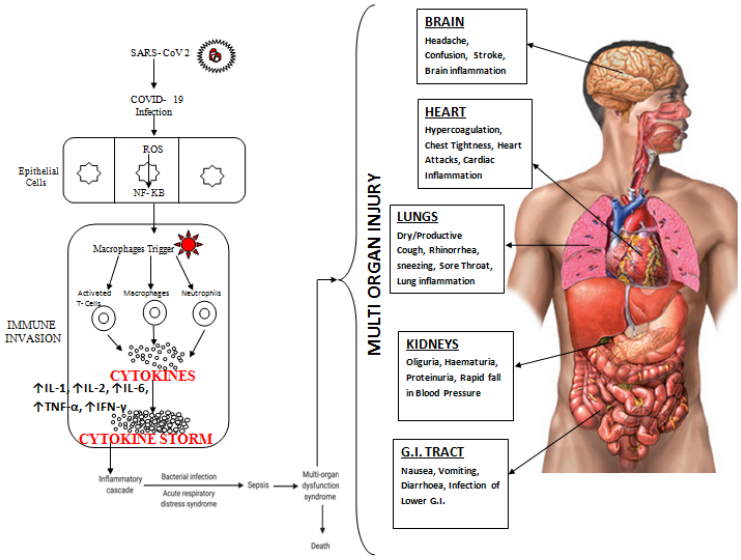


Fig 1: Mechanisms of SARS-CoV-2 associated cytokine storm. Infection with SARS-CoV 2 can stimulate a hyperinflammatory immune response wherein epithelial-cell-mediated production of reactive oxygen species (ROS) can also stimulate the synthesis of NF-κB which contribute to increased cytokine levels of ↑IL-1, ↑IL-2, ↑IL-6, ↑TNF-α, ↑IFN-γ **(CYTOKINE STORM)**. This essentially causes immune invasion which can lead to clinically relevant conditions such as ARDS, sepsis, multiple organ dysfunction syndrome(MODS) & death. All organs (like heart, lungs, kidneys, brain & G.I. tract) having ACE-2 receptors are always vulnerable to SARS-CoV2 infection.

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; ROS, reactive oxygen species; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; MODS, multiple organ dysfunction syndrome.

COX-2 plays a dual role in inflammatory process, i.e. pro-inflammatory and anti-inflammatory (Resolution). In the inflammatory phase COX-2 promotes generation of pro-inflammatory lipid mediators from Arachidonic Acid like prostaglandins (PGs) and leukotrienes (LTs) and in the anti- inflammatory phase, it promotes generation of pro- resolvins from Omega- 3 fatty acid nutrients (EPA & DHA). This depends upon presence or absence of aspirin. EPA + DHA are the necessary building blocks of resolvins. On the contrary, Omega- 6 (AA) generates pro- inflammatory eicosanoids. Using the same COX and LOX enzymes operative during the initiation of acute inflammation, the host is able to shift the chemical mediator profile from pro-inflammatory to pro-resolving by modulating biosynthetic pathways to induce 15-LOX expression and activity to generate LXs, RVs and PD1.

Resolvins are specialized pro-resolving lipid autacoids mediators (SPMs). They are divided into two classes i.e. EPA derived E- series (RvE1, RvE2, & RvE3) & DHA derived D- series (RvD1, RvD2 & RvD3) (Fig 2) (14). Resolvins reduce inflammation by blocking trans endothelial migration, regulate interleukin 12 production & lead to resolution of the inflammatory response to resolve inflammation. Resolvin molecules do not inhibit the onset of inflammation but stimulate its resolution pathways. They promote the resolution of the inflammatory cycle. They also stimulate the phagocytosis of apoptotic PMNs by macrophages and selectively disrupt thromboxane-mediated platelet aggregation.

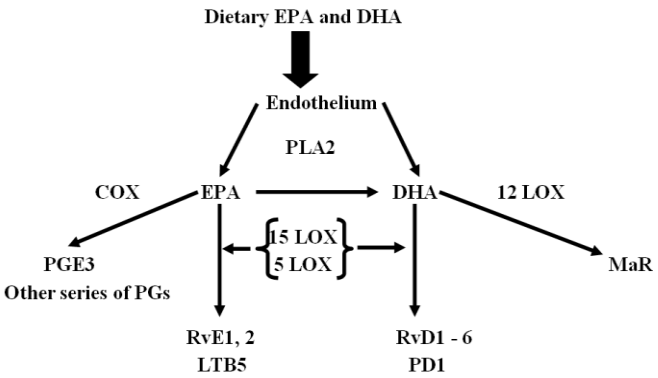


Fig 2: Formation of anti-inflammatory metabolites by metabolism of DHA and EPA. These metabolites bind to their respective receptors & elicit anti-inflammatory changes in cells. It leads to decrease in IL-6, IL-1, or TNFα, key cytokines provoking cytokine storm. (PLA2, phospholipase A2; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LOX, Lipoxygenase; PGs, Prostaglandins; PGE3, Prostaglandin E3; RvE1,2, Resolvin E1 & E2; LTB5, Leukotriene B5; RvD1-6, D-series resolvins; PD1, Protectin D1; MaR, Maresin)

Hyperinflammation (Cytokine Storm) is the result of much higher production of proinflammatory mediators (IL-6, IL-1β, TNF-α etc.) than proresolvins to resolve inflammation. Deficit in resolution result in hyperinflammation.

2.3 COVID-19 ASSOCIATED COAGULOPATHY (CAC)

The main underlying mechanism responsible for higher coagulation tendency during COVID-19 is over- activation of immune system causing complement release syndrome. Elevated IL-6 the immunomodulator is the trigger factor for coagulation disorders (15).

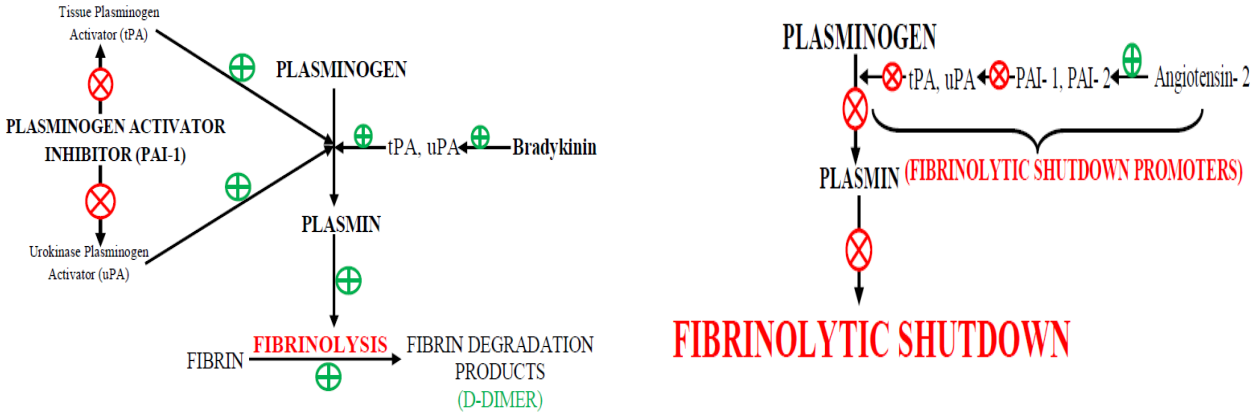
SARS- COV-2 induced infection can lead to arterial & venous thromboembolic events by either inducing excessive systemic inflammatory response, pro-coagulant activity, immobilization, & hypoxia, or causing DIC (16, 17 and 18). DIC is characterized by systemic intravascular activation of coagulation, leading to the deposition of fibrin & formation of widespread micro vascular thrombosis. Pulmonary intravascular coagulation (PIC) is distinct from DIC as it is immune thrombosis (19).

Elevated levels of fibrinogen and D-dimer & mild prolongation in PT/aPTT were seen in patients with CAC. This correlates with a parallel rise in markers of inflammation (e.g. CRP) unlike the pattern seen in classic DIC from bacterial sepsis or trauma, prolongation of the aPTT and/or PT is minimal, thrombocytopenia is mild (platelet count ~100 $x10^9$/L) (Table 1) (20).

Table 1: DIFFERENCE BETWEEN CAC & DIC

FEATURE	COVID-19 ASSOCIATED COAGULOPATHY (CAC)	DIC
PULMONARY INVOLVEMENT	++++	++
BLEEDING	UNCOMMON	PROMINENT
THROMBOSIS	++	+
THROMBOCYTOPENIA	+	++
PT/APTT PROLONGATION	MILD, COMMON	MARKED, VERY COMMON
FIBRINOGEN	INCREASED	DECREASED
D-DIMER	++++	++

2.4 FIBRINOLYTIC SHUTDOWN IN COVID- 19



(A) (B)

Fig 3 : (A) Generation of plasmin, the fibrinolytic enzyme, follows the activation of plasminogen by plasminogen activators (PA; tissue-type PA [tPA] & urokinase-type PA [uPA]), which are themselves inhibited by type 1 plasminogen activator inhibitor (PAI-1). The enzymatic activity of plasmin is inhibited by 2-antiplasmin (2-AP; & potentially by 2-macroglobulin) (21). (B) The direct infection of endothelial cells by the virus (a mechanism that is rather specific for coronaviruses via their cell entry through ACE2 (angiotensin-converting enzyme 2, the receptor for SARS-CoV-2), abundantly expressed on endothelium) leads to a massive release of plasminogen activators (22). ACE2 helps to mediate anticoagulant properties of the vascular endothelium in the healthy state, binding of SARS-CoV-2 to ACE2 aggravates cell damage, upregulates tissue factor expression (23). SARS- CoV- 2 binds to the ACE -2 Receptor which is an important Component for Renin Angiotensin System. Due to overusage of ACE 2, it will result in excess of Angiotensin 2 which is stimulator of PAI-1; this inhibits the action of Plasminogen activator which helps in fibrinolysis by converting Plasminogen to Plasmin.

Excessive coagulation with fibrinolytic shutdown is the hallmark of CAC (Fig 3). This is a big challenge to treat critical COVID- 19 patients. Best option is to check its progression right from mild stage to critical one.

2.5 TISSUE FACTOR- LIFE THREATENING MEDIATOR OF CAC

Cytokine storm induced over expression of Tissue Factor is the potential trigger for CAC. Tissues of lungs, brain & surface of cells like endothelial cells, macrophages & monocytes store tissue factor (TF). Normally they are not in circulation. But after vascular injury, they get exposed to blood & platelets. Cytokines like IL-1, TNFα & endotoxins help their release in blood which makes it critical mediator leading to easy & fast development to CAC. TF binds with activated factor VIIa when exposed to both platelets & blood. They then promote the activation of factor IX & X to IXa & Xa, respectively, ultimately results in common coagulation pathway & the consequent formation of both thrombin & fibrin (Fig 4) (24, 25, and 26). These micro thrombi impair organ perfusion & in turn lead to organ failure. Increased activity of coagulation cascade results in the formation of more fibrin clots in blood which finally leads to the deposition of platelets on it & hence, increasing the size of these formed clots. Large clots will result in blockage of blood vessels & cause life threatening embolism due to CAC. During the whole process, coagulation inhibitors get used up which further worsens this ongoing condition because reduced inhibition will lead to increased coagulation. Elevations in fibrinogen levels & D- dimer levels are also observed. This correlates with a simultaneous increase in inflammatory markers (e.g. CRP).

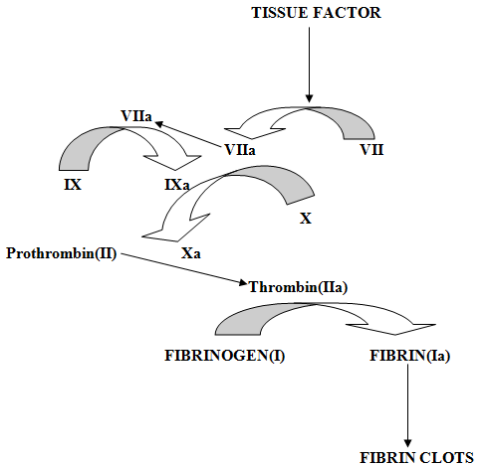


Fig 4:- Tissue factor (TF) (also called as platelet tissue factor, factor III & CD142) released in response to the cytokines like IL-1, TNFα & endotoxin leads to coagulation. Increase in tissue factor results in activation of coagulation cascade by activating factor VII to VIIa which further activates other coagulation factors like IX & X to IXa & Xa respectively & subsequently form thrombin & fibrin.

3. ASPIRIN IN COVID- 19

Aspirin is found to have a lot of pharmacological properties due to its binding & inhibition of COX enzyme & many other pharmacologically active sites (27). Aspirin is useful as an antipyretic & analgesic. But the antithrombotic & anti- clotting property made it distinct from other popular NSAIDs like Ibuprofen or paracetamol. This property has also attracted the interest of many scientists to find its potential benefits & provide a supportive therapy for COVID-19 induced

coagulopathy. Moreover, it also reduces the symptoms like pain & fever during SARS-CoV2 infection. Also it can stop progression of cases to life threatening stage by its anti-inflammatory, antithrombotic & antiviral effects (27, 28, 29, 30, 31 and 32).

3.1 ASPIRIN – AN ANTIVIRAL

Aspirin used in an in-vitro study has shown an antiviral effect on other previous human coronavirus like MERS CoV & SARS CoV. It impairs the progression of these viruses by inhibiting virus induced NF-kB activity during replication, resulting in reduced number of copies & act as a virostatic agent in smooth muscles (33, 34). Aspirin got a lot of popularity during the last pandemic of Spanish flu in 1918. In a study Aspirin was found to be effective against influenza virus by inhibiting prostaglandin E2 (PGE2) in macrophages & increased the production of interferon type 1(INF-1), results in reduced viral multiplication & increased immune response governed by T-cells (33, 34).

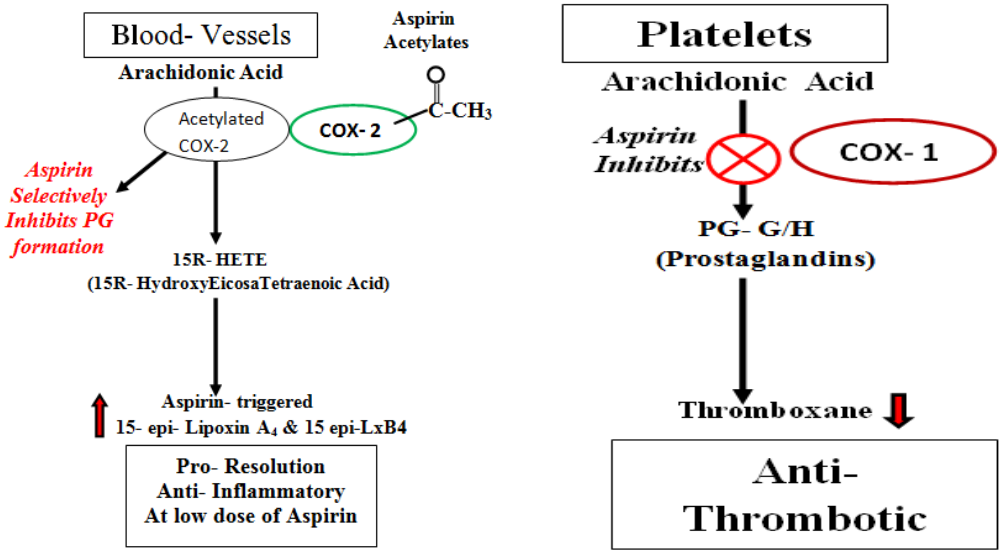


Fig. 5: Role of Aspirin in (a) Thromboinflammation and (b) Antithrombotic

3.2 ASPIRIN IN THROMBOINFLAMMATION

Distinct series of pro-resolving lipid mediators are generated, depending on the parent substrate and the presence or absence of aspirin. Expression of COX-2 is increased during acute inflammation (35) and the ingestion of aspirin leads to acetylation of COX-2, which blocks PG formation (36). COX- 2 initiation of leukotriene synthesis is prevented by acetylation (37, 38). Acetylated COX-2 is not catalytically inactive, converts arachidonic acid to 15(R)-HETE rather than PGs (39). 15(R)-HETE can serve as a substrate for 5-LOX for transformation to 15(R)-LXA4. Aspirin-triggered 15-epi-LXs are ~twofold more potent than 15(S)-LXs (13). Aspirin can trigger the synthesis of stereoisomers (epimers) of LXA4 & LXB4 (these compounds are also referred to as Aspirin-Triggered Lipoxins (ATLs)). The biochemical synthesis of these protective compounds is enzymatically mediated (Fig. 5 (a)) .

Aspirin is unique among other NSAIDs because it irreversibly inhibits COX-2 by acetylation of an amino acid serine residue preventing prostanoid generation yet enabling the biosynthesis of endogenous anti-inflammatory mediators. Low-dose ASA triggers the resolution phase by activating endogenous epimers of specialized pro-resolving lipid mediators in humans. Low-dose ASA may be considered “resolution friendly” since it mimics endogenous biosynthetic mechanisms to trigger new mediators, leading to a favourable net change for pro-resolution.

3.3 ASPIRIN - ANTITHROMBOTIC

Aspirin is the most trusted antiplatelet drug because of its high selectivity to COX-1 inhibition (40). According to the study, Inhibition of COX-1 by aspirin is 10 times greater than that of COX-2 (IC_{50} 3.5 v/s IC_{50} 30 µM) (41, 42 and 43). Aspirin at lower dose of 75-100 mg irreversibly acetylate Ser530 site of COX-1 & inhibits formation of TXA_2, a vasoconstrictor & platelet aggregator (44). Aspirin induced inhibition of TXA_2 makes it a significant antithrombotic agent (Fig 5(b)) (45).

4. RISK FACTORS IN CAC

Pharmacological properties of aspirin, like anti-inflammatory, antithrombotic and antiviral can be used as an evidence to hypothesize its use as a primary preventive treatment for CAC. But rising trend in aspirin resistance has made aspirin totally undependable. Restoring aspirin sensitivity to its original status may be useful as a treatment protocol in coagulopathy especially in CAC.

4.1 ASPIRIN RESISTANCE- CAUSE OF CONCERN:

Aspirin resistance is an inactivity of aspirin to decrease TXA_2 formation & in turn platelet aggregation & activation. Aspirin resistance doesn't refer to an absence of expected antiplatelet activity of aspirin only but also poor clinical outcomes, like 3 fold higher risk of death, heart attack & stroke (46).

Aspirin resistance was defined by the presence of at least 2 of the following 3 criteria: 0.5-mg/ml AA-induced platelet aggregation >20%, 10 µmol/l ADP-induced aggregation >70% & ARU >550 (in contrast to the screening process in which an ARU {Aspirin Reaction Unit} >500 was used as the cutoff) (47). Time tested activities of aspirin can prove to be useful in COVID-19. The main hindrance in aspirin usage is high prevalence of aspirin resistance among mass population i.e. 24.75% in CVD patients as per WHO cardiovascular disease (CVD) factsheet 2017 (48). According to another study its prevalence is 5-75% (49) & 38.1% in Indian patients with documented heart disease (50) .Up to 40% of patients with CVD do not comply with Aspirin (51).

4.2 LOW OMEGA-3 INDEX

The **Omega-3 Index** test is simply a measure of the amount of EPA & DHA in the blood, specifically the red blood cell membranes. For example, if you have 68 fatty acids in a cell membrane & 4 are EPA & DHA, then you would have an **Omega-3 Index** of 5.88%. When you take an **Omega-3 Index** test it gives you a percentage. Low omega-3 index (≤ 4%) is an independent risk factor for CHD mortality (52).Whereas, ≥8% is cardioprotective (53). Low Omega- 3 index reflects high Omega- 6/ Omega- 3 ratio.

4.3 OMEGA-6: A PROCOAGULANT:

Omega-6 fatty acid (AA) is the substrate for the synthesis of a variety of proinflammatory/ proaggregatory molecule such as prostaglandin E_2, thromboxane A_2 (TXA_2), & leukotriene B_4. High omega-6 concentration overproduce them leading to coagulation (54). TXA_2 has prothrombic properties, as it stimulates the activation of platelets & platelet aggregation. TXA_2 is also a known vasoconstrictor & gets activated during times of tissue injury & inflammation (55). Leukotriene B_4, a powerful inducer of leukocyte chemotaxis & adherence also induce coagulation.

4.4 HIGH OMEGA-6/OMEGA-3 FA RATIO IN WORST AFFECTED COVID-19 COUNTRIES:

High Omega-6/Omega-3 ratio shifts the balance into pro-inflammatory/pro-aggregatory state. Data collected from various sources confirm the high prevalence of omega-6/omega-3 ratio (15- 17/1) in almost all worst affected countries (Table 2).

ESSENTIAL FATTY ACID STATUS

Table 2: Table showing country-wise total number of COVID- 19 cases till 8th October, 2020

S.No.	COUNTRY	COVID- 19 Cases	Omega- 3 Index	Omega-6/ Omega-3 Ratio
1	**U. S. A.**	77,76,796	≤ 4%	**15 - 17/1**
2	**India**	68,35,655	≤ 4%	**37/1 ****
3	**Brazil**	50,02,357	≤ 4%	**15 - 17/1**
4	**Russia**	12,60,112	> 4 to 6%	**(6.39/1)***
5	**Spain**	8,72,276	> 4 to 6%	**15 - 17/1**
6	**Mexico**	7,99,188	N/A	**15 - 17/1**
7	**France**	6,53,509	> 4 to 6%	**15 - 17/1**
8	**U. K.**	5,44,275	≤ 4%	**15 - 17/1**
9	**Iran**	4,83,844	≤ 4%	**NA**
10	**Italy**	3,33,940	≤ 4%	**15 - 17/1**
11	**Germany**	3,11,113	> 4 to 6%	**15 - 17/1**

**As per Urban Indian Diet (56).

* Calculated from omega-6 & omega-3 intake {Total omega-6(LA+AA)/Total omega-3(ALA+EPA+DHA)} (57).

5. MANAGEMENT OF ASPIRIN RESISTANCE, LOW OMEGA- 3 INDEX & HIGH OMEGA-6/ OMEGA- 3 RATIO

5.1 ASPIRIN RESISTANCE MANAGEMENT

Aspirin resistance problem can only be solved by minimizing thromboxane A_2 production by co-administration of another substance with aspirin which could manage to reduce the TXA_2 production. This can be achieved with co-administration of EPA & DHA with aspirin. Their supplementation helps in replacing omega 6 fatty acids (such as AA). This increase in EPA/AA ratio will ultimately help in reduction of TXA_2. There are various studies which have shown a synergistic effect of low dose aspirin with EPA & DHA supplementation. These studies have proved that this combination is an effective antithrombotic treatment. In a study TXB_2 (inactive metabolite of TXA_2) was evaluated & reduction of 56.8% with EPA, DHA & low dose aspirin in 30 days were reported (58). Aspirin's antiplatelet effect is also enhanced by omega-3 fatty acids. Increase in bleeding time by 33% is also observed in 11 weeks due to TXA_2 inhibition (59). In another study combination of aspirin, EPA & DHA have shown positive result as antiplatelet but as individual they are not much effective (60). The effects of aspirin and fish oil consumption on lysophosphatidylcholines and lysophosphatidic acids and their correlates with platelet aggregation in adults with diabetes mellitus (61).

5.2 TISSUE FACTOR MANAGEMENT

In a dose dependent study (3-4g EPA + DHA/d v/s 1g EPA + DHA/d) there was significant 7.5 fold decrease in tissue factor (-5.3vs -0.7), 22 times in IL-6 and 2 times in TNF-α at higher doses (62).

5.3 LOW OMEGA-3 INDEX MANAGEMENT

Dietary omega-3 fatty acids like DHA reduce inflammatory eicosanoid production from Arachidonic Acid (AA) (63), adhesion molecules (VCAM-1 & ICAM-1) (64), chemokines (MCP1) (65), matrix metalloproteinases & inflammatory cytokines (66). Mammalian cells lack conversion of omega-6 to omega-3 fatty acids because of the absence of converting enzyme, i.e., omega-3 desaturase (State of high Omega-6/ Omega-3 ratio). This condition is associated with increased production of thromboxane A2 (TXA_2), leukotriene-B4 (LTB-4), IL-1, IL-6, tumor necrosis factor (TNF) & C-reactive protein which increase by increased intake of omega-6 fatty acid & decrease by increased intake of EPA & DHA. Omega-3 PUFAs for 4 weeks lowered fibrinogen, thrombin & factor V levels (67). 2 to 3 grams of EPA per day for 11 weeks prolonged bleeding time by 33% (68). High-dose (1.5 g/day EPA + 1.0 g/day DHA) n-3 supplementation can reduce plasma levels of both IL-6 and IL-1ß (69). The anti-inflammatory effect of EPA and DHA supplementation seems consistent (70).

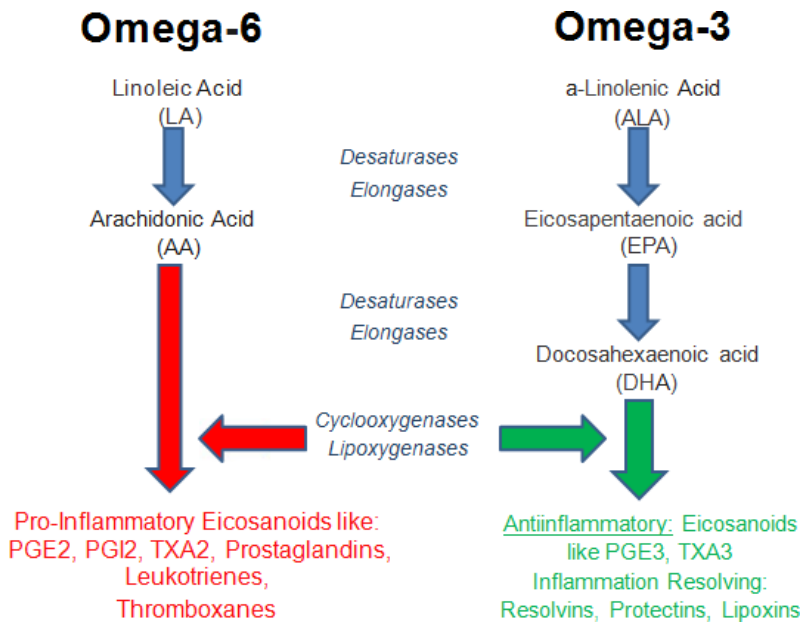


Fig 6: Biological & metabolic roles of omega-6 & omega-3 fatty acids.

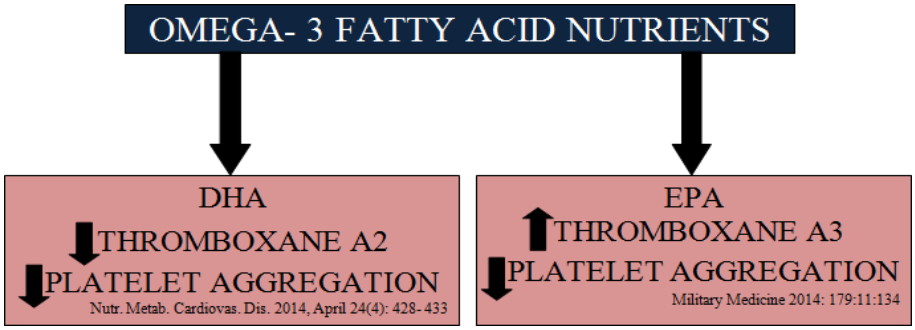


Fig 7: Role of Omega-3 nutrients as anti-platelet aggregators. DHA downregulates(⬇) Thromboxane A2 whereas, EPA upregulates(⬆) Thromboxane A3, both of which reduces platelet aggregation. Also EPA derived Prostacyclin I 3 reduces chances of bleeding (71, 72).

Eating more EPA+DHA can prevent chronic low-grade inflammation (73). Both EPA & DHA have anti-inflammatory benefits (Fig 6) (73). Both EPA & DHA inhibit platelet aggregation (74, 75). They directly & indirectly reduces the formation of the AA proaggregatory metabolite TXA_2 (Fig 7) (76). The assimilation of omega-3s in red blood cells seems to reduce whole blood viscosity & increase red blood cell flexibility thus reduces the risk of thrombosis (74, 75). Daily supplementation with 3 g of EPA/DHA for 18 weeks, showed anti- thrombotic effects of omega-3s in clinical studies in healthy patients. The long chain omega-3 PUFAs EPA & DHA have anti-platelet effect (77).

5.4 RESOLUTION OF THROMBOINFLAMMATION

Omega- 3 fatty acids (EPA + DHA) supplementation exerts their anti- inflammatory actions by competing with omega- 6 (Arachidonic Acid), reducing pro- inflammatory eicosanoids and increasing proresolvins (Fig 8) (78). Aspirin induces synthesis of Resolvins & the Protectins with the help of EPA and DHA (79, 80, 81 and 82). A combination of aspirin & omega-3 PUFAs (EPA + DHA) is an effective prevention strategy in cardiovascular diseases. Aspirin, EPA and DHA combination not only prevents the onset of inflammation but also resolves it (83).

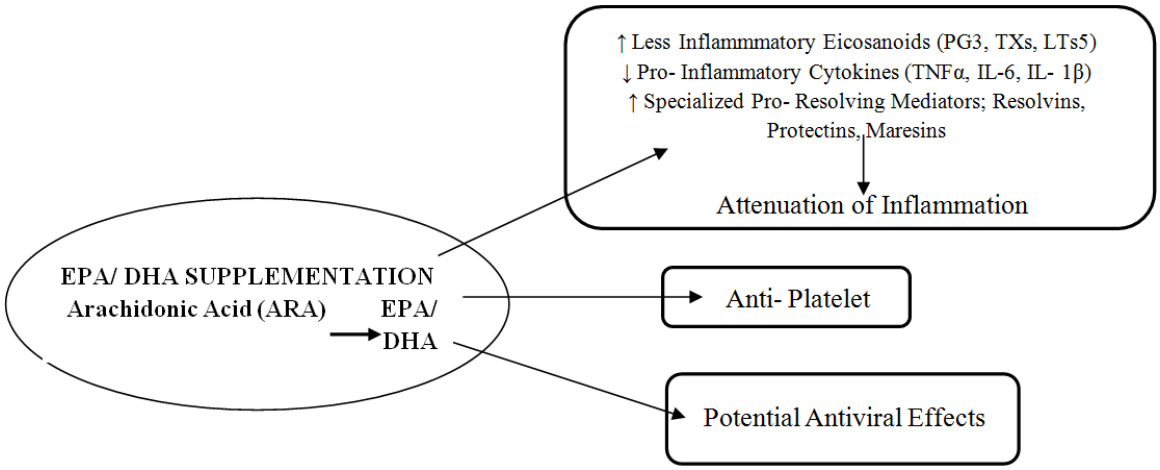


Fig. 8: Role of EPA + DHA in Resolution of Thromboinflammation

So activation of body's own resolution pathways with low dose aspirin + EPA + DHA may become novel therapeutic approach to limit thromboembolism in CVD and especially CAC and improve outcome in severe COVID-19 patients (84).

6. CONCLUSION

To identify relevant studies, a comprehensive literature search was conducted. Supplementary literature searches included examining the reference lists of all relevant studies, pertinent review articles, & meta- analyses. Our objective was to systematically review the available literature & to identity the relationship between aspirin resistance, low omega- 3 index (EPA + DHA levels), high omega-6/omega-3ratio & COVID- 19 induced coagulation. WHO has stated that there should be an optimal balance in the dietary intake constituted by n-6 & n-3 PUFA (85). However, n-6 PUFA are found in large

amounts in most plant oils & its consumption has exploded in the past decades while fish consumption has steadily declined (86). Over-supply of omega-6 PUFAs and under-supply of omega-3 nutrients lead to striking imbalance between these two. This decreases omega- 3 index and increases omega- 6/ omega- 3 ratio. This can substantially increase the chances of thromboinflammation.

According to various studies aspirin resistance is quite common in aspirin taking patients. So we hypothesize that, aspirin resistance and low omega- 3 index in CVD patients can trigger coagulopathy specifically CAC in COVID- 19. Hence co-administration of another substance which can reduce TXA_2 is required to potentiate aspirin's anti-thrombotic actions and overcome aspirin resistance. Adequate intake of 3- 4 grams/ day of EPA + DHA not only meet these requirements but also increases omega- 3 index and reduces omega- 6/ omega- 3 ratios. This along with low dose aspirin can help in resolving cytokine storm and check progression of COVID- 19 to severe stage. Resolving inflammation with aspirin and resolvins producing EPA + DHA is a better and effective way to subside cytokine storm than COX-2 inhibitors like NSAIDs.

We don't claim that high omega-6/omega-3 ratio is the only contributory factor causing high mortality. It can be one of the major factor besides median age, immunity etc. To the best of our knowledge this is the first study correlating aspirin resistance and low omega-3 index i.e., high omega-6/omega-3 ratio with severity and mortality in COVID-19 patients. Adequate supplementation of omega- 3 nutrients i.e., EPA & DHA may help in correcting this harmful imbalance. We propose that there is a need for further research and clinical trials to establish this hypothesis of combined therapy of aspirin, EPA and DHA. This can improve both aspirin efficacy as well as Omega-3 index resulting in decreased Omega-6/Omega-3 ratio. This might help in keeping COVID-19 symptoms in mild stage & check their progression to life threatening CAC.

ABBREVIATIONS:

S. No.	ABBREVIATIONS	EXPLANATION
1.	CVD	Cardiovascular Disease
2.	COVID-19	CoronaVirus Disease of 2019.
3.	CAC	COVID-19 Associated Coagulopathy
4.	aPTT	Activated Partial Thromboplastin Time
5.	PT	Prothrombin Time
6.	SARS CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
7.	ACE-2	Angiotensin-Converting Enzyme 2
8.	RNA	Ribonucleic Acid
9.	IL	Interleukin
10.	TNF-α	Tumour Necrosis Factor- α
11.	ROS	Reactive Oxygen Species
12.	IFN-γ	Interferon Gamma
13.	ARDS	Acute Respiratory Distress Syndrome
14.	MODS	Multiple Organ Dysfunction Syndrome
15.	COX	Cycloxygenase
16.	PG	Prostaglandins
17.	LT	Leukotrienes
18.	EPA	Eicosapentaenoic Acid
19.	DHA	Docosahexaenoic Acid
20.	LOX	Lipoxygenase
21.	LXs	Lipoxins
22.	RVs	Resolvins
23.	PD1	Protectin
24.	SPMs	Specialized Pro-Resolving Lipid Autacoids Mediators
25.	PLA2	Phospholipase A2
26.	DIC	Disseminated Intravascular Coagulation
27.	PIC	Pulmonary Intravascular Coagulation
28.	CRP	C-Reactive Protein
29.	PA	Plasminogen Activator
30.	tPA	Tissue Type Plasminogen Activator
31.	uPA	Urokinase Type Plasminogen Activator
32.	PAI-1	Plasminogen Activator Inhibitor-1
33.	AP	Antiplasmin

34.	TF	Tissue Factor
35.	HETE	Hydroxyeicosatetraenoic Acid
36.	LXA4	Lipoxin A4
37.	ATLs	Aspirin Triggered Lipoxins
38.	ASA	Acetyl Salicylic Acid
39.	IC_{50}	Half Maximal Inhibitory Concentration
40.	TXA_2	Thromboxane A2
41.	ADP	Adenosine Diphosphate
42.	ARU	Aspirin Reaction Unit
43.	WHO	World Health Organisation
44.	CHD	Coronary Heart Disease
45.	CAD	Coronary Artery Disease
46.	LA	Linolenic Acid
47.	ALA	Alpha-Linolenic Acid
48.	TXB_2	Thromboxane B2
49.	VCAM-1	Vascular Cell Adhesion Molecule 1
50.	ICAM-1	Intercellular Adhesion Molecule 1
51.	MCP1	Monocyte Chemoattractant Protein-1
52.	PUFAs	Poly Unsaturated Fatty Acids
53.	NSAIDs	Non Steroidal Anti-Inflammatory Drugs

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