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# Elevated broad spectrum protease inhibitor, alpha-2-macroglobulin (A2M) in long lived naked mole rat (NMR) may open a new window to develop a medicine for our healthy life

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**Abstract**

The discovery of the life pattern of long lived NMR (Heterocephalusglaber) may be a milestone to develop a new concept "how we can live long with a healthy life". Recently it was reported that, in NMR, elevated level of a broad spectrum protease inhibitor, A2M plays an important role in anti-cancer and anti-aging mechanisms. Raising A2M level in our body by eating "some natural products" may be a milestone in developing a method (A2M-ShopAnn System) for prevention and treatment of many disease(s). Since A2M (a physiological broad spectrum protease inhibitor) is continuously eliminating proteases and other toxic products from our body and to keeping us healthy, by measuring A2M concentration in our body can be speculated the current status of the body in preventing capacity of any individual from the pathological effect of protease(s) and other toxic products which are the key elements for the beginning of many disease(s).

**Keywords:** naked mole rat, alpha-2-macroglobulin, protease, medicine, disease, A2M-ShopAnn System self-care

The amount of A2M in NMR is nearly double the amount in humans ( Thieme et al. 2015) indicated that inhibition of harmful effects of proteases(s) may prevent the onset of many diseases. The pathological effects of protease(s) and their involvement in the disease process (s) are well known (Watanabe et al., 1995 May; Edinger, et al. 2015; Almeida-Paes et al. 2015; da Silva Júnior et al. 2015; Chinello et. el. 2015 ; Raut et al. 2015, Gowrishankar et al. 2015; Påhlman et al. 2015). Based on this concept, many protease inhibitors were also developed and are being used to treat many diseases for long time in clinical fields (Table 1) (Leung et. al. 2000).

**Table: 1**

*Serine protease inhibitors in clinic*:

| Protease | Function | Disease | Drug/Status |
|---|---|---|---|
| Thrombin | Blood coagulation | Stroke | Argtroban, bivalirudin |
| | | Coronary | Ximelagartan, Melagatran |

Vol. 2, No. 03; 2018

ISSN: 2581-3366

| | | | |
|---|---|---|---|
| | | infarction | |
| Factor Xa | Blood coagulation | | Danaparoid (launched), |
| | | | DX-9065a, CI-1031(Phase-II trial) |
| | | | DPC-906, JTV-803(Phase-II trial) |
| | | | MLN-1021,PMD-3112(Phase-I trial) |
| Factor VIIa | | | NAPc2 (Phase-I trial) |
| HNE elastase | Cleaves elastin | SIRS | Sivelestat (Japan only) |
| | | ARDS | Sivelestat (Phase-II trial, USA) |
| Complement | Inhibition | Inflammation | Nafamostat, FUT-175 |
| HCV | HCV repication | Hepatitic C | BLIN-2061 (Phase-II trial) |
| | | | VX-950(Preclinical) |
| PAI (Urokinase) | | Cancer | WX U K1 (Phase II) |
| | | | Aminocaproic Acid (Phase III) |
| | | Ulcer, Psoriasis | PAI-2 (Phase-II trial) |
| Matriptase | | Prostate Cancer | CVS-3983 |
| Chymase | Restenosis | | NK-3201 (preclinical) |
| Dipeptydil Peptidase IV | | Diabetes type II | LAF-237, P32/98 (Phase-II trial) |
| *Aspartic protease inhibitors in clinic*: | | | |
| **Protease** | **Function** | **Disease** | **Drug/Status** |
| HIV-1 protease | HIV replication | HIV/AIDS | Indinavir, Nelfinavir, |
| | | | Amprinavir, Ritonavir, |
| | | | Lopinavir, Saquinavir, |
| | | | Atazanavir, Fosamprenavir. |
| | | | Tipranavir (Phase III) |
| | | | (monotherapy unsuccessful) |
| Renin | forms Angiotensin 1 | Hypertension | Aliskiren (Phase II) |
| BASE | forms $A\beta_4$ | Alzheimers | Elan (Preclinical) |
| | | | Actelion (Preclinical) |
| | | | Locus (Preclinical) |
| | | | TGCN-001(Preclinical) |
| | | | Astex Technology (Preclinical) |

Vol. 2, No. 03; 2018

ISSN: 2581-3366

| | | | |
|---|---|---|---|
| | | | Sunesi s (Preclinical) |
| | | | De Novo (Preclinical) |
| *Cysteine protease inhibitors in clinic*: | | | |
| **Protease** | **Function** | **Disease** | **Drug/Status** |
| Rhinovirus | Viral replication | SARS | Ruprintrivir(preclinical) |
| SARS CoV M | Viral replication | SARS | AG7088(preclinical) |
| Cathepsin K | Bone resorption | Osteoporesis | AEE-58,SB-462795(Phase-II) |
| Caspase 1 | Cytokinine release | Arthritis | VX-765 (Phase-1) |
| | | | Pralnacasan (phase II) |
| Caspase 3 | Apoptosis | Cancer, Alzhemier | Locus Pharma |
| | | Ischaemia, Sepsis | Novartis (preclinical) |
| Caspase 8 | Apoptosis | Sepsis, Diabetes | IDN-6556 (Phase II) |
| Cruzain | parasitic replication | Trypanosomiasis | K-777, INPL-022-E7 (preclinical) |
| Cathepsin F, L, S | | | INPL-022-E7, D6 (preclinical) |
| *Metallo protease inhibitors in clinic*: | | | |
| **Protease** | **Function** | **Disease** | **Drug/Status** |
| ACE-1 | Forms Angiotensin-II | Hypertension | Trandolapril, Enalapril, Captopril |
| NEP | Release of ANP | Hypertension | Candroxatril (discontinued) |
| TACE | Release of TNFα | Arthritis, MS | BMS-561392 (Phase-II) |
| MMP-1 | Degrades matrix | Cancer | Marimistat(discontinued), |
| | | | Neovastat (Phase-III) |
| | | Periodontitis | Periostat |
| MMP-2 | | Cancer | Rebimistat(Phase-1) |
| MMP-8 | | Osteoarthritis | Glocosamine sulphate |
| MMP-9 | | Inflammation | Rega-3G12, Biopharma |
| MMP-3,13 | | | Pfizer, Novartis |

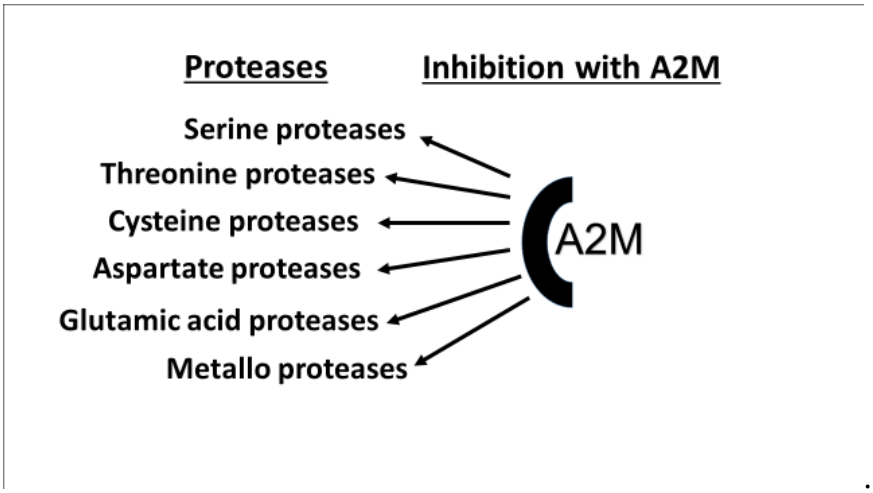
Vol. 2, No. 03; 2018

ISSN: 2581-3366

| | | | |
|---|---|---|---|
| | | | (preclinical) |
| *Threonine protease inhibitors in clinic*: | | | |
| **Protease** | **Function** | **Disease** | **Drug/Status** |
| Proteosome | | Ischaemia | Bortezomib, MLN-519 (Phasel) |

Targeting drugs against any individual type of protease, may be less effective and less successful in the treatment of disease(s) where proteases are involved. But targeting all types of proteases by using a single broad spectrum protease inhibitor, A2M, may be more beneficial in the treatment of many diseases (Figure 1) (khan MM et al. 2017).

Figure 1.




A2M inhibits not only protease(s), it is also continuously removing redundant chemicals from our body, such as growth factors; cytokines; hormones; soluble beta-amyloid etc. which causes many diseases (Khan MM et al. 2017; Khan MM et al. 2016; Armstrong 2006).

Proteins of the A2M family are present in a variety of animal phyla, including the nematodes, arthropods, mollusks, echinoderms, urochordates, and vertebrates and invertebrates or even plant kingdom (Armstrong 2006). A shared suite of unique functional characteristics have been documented for the A2Ms of vertebrates, arthropods, and mollusks. The A2Ms of nematodes, arthropods, mollusks show significant sequence identity in key functional domains. Thus, the A2Ms comprise an evolutionarily conserved arm of the innate immune system with similar structure and function in animal phyla separated by 0.6 billion years of evolution(Armstrong 2006). A2M prevents protease induced activation of coagulation by inhibiting thrombin and inhibitor of fibrinolysis by inhibiting plasminand kallikrein. Numerous growth factors, cytokines and hormones bind to A2M. A2M also binds soluble beta-amyloid, of

Vol. 2, No. 03; 2018

ISSN: 2581-3366

which it mediates degradation as seen in the brain of Alzheimer's disease (AD) patients. A2M is synthesized mainly in liver, but also locally by macrophages, fibroblasts, and adrenocortical cells.A2M is also produced in the brain where it binds multiple extracellular ligands and is internalized by neurons and astrocytes (Nezu et al. 2013; Zhang et al. 2007; Bätz et al. 2014; Borisova et al. 2014; Marino et al. 2002; Hammond et al. 2005).

Previously we have shown that A2M is the major innate immune factor against foreign protease and exogenous A2M is a major life saving protein in the critical stage of protease induced septic shock animal models (Khan & Scharko. 2012; Khan et al. 1993a; Khan et al. 1994; Khan et al. 1995; Puente et al. 2003; Seife et al. 1997; Watanabe et al. 1995). In animal research it was clearly documented that in a Gram negative bacterial septic shock model, level of A2M went down to 30% of its original concentration and remaining 30% of A2M was not enough to protect animals from death. In that study, injecting purified A2M at the very crisis stage of septic shock was able to rescue animals from death which worked as a life-saving medicine(Khan et al. 1993a; Khan et al. 1994; Khan et al. 1995).

In the description of innate immune system the role of A2M had never been described in medical immunology. Invasion of pathogen into the body is the beginning of many disease processes. Micro-organisms secrets proteases for invasion into our body but A2M in our blood (acts as guards)normally to prevent the protease activity and keeping us healthy (Khan 2016) (Fig 2).

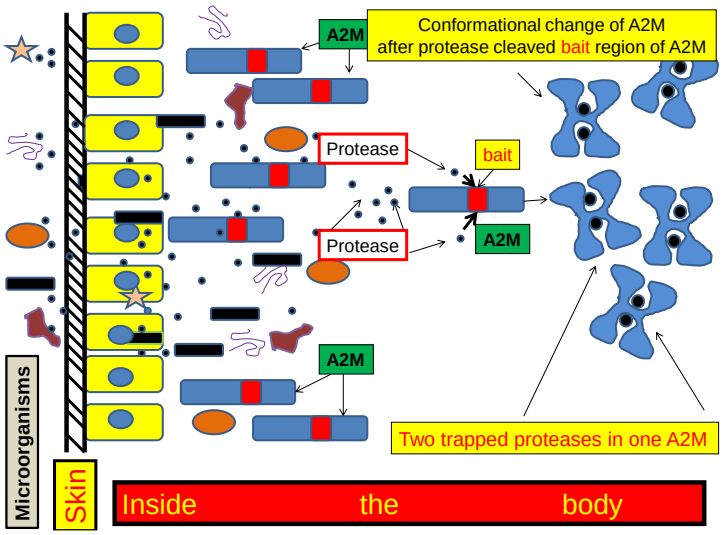



Fig 2. A schematic diagram of different kind of proteases (●) and A2M( ) interaction. Microorganisms ( , , , , ) invade the skin or endothelial surface by using proteases (●) . Once they are inside the body they continue to release proteases and start activating other systems (such as: coagulation, complement, Interleukin etc. and many others). Proteases binding and cleaving the 35 amino acid “bait

Vol. 2, No. 03; 2018

ISSN: 2581-3366

region-red spot" (  ) become bound proteinase-A2M complex (  ). Probably, this is the first line of defense by A2M in our innate immune system.

Therefore, A2M could be considered as the first major component for defense in the innate immune system.

The status of A2M concentration in the body may be a very important predictor for the severity of many diseases. Normally many toxic products, in addition to proteases also binds with A2M and removed from the body. Deficiency of A2M from its physiological concentration is not only risk for protease clearance, but also risk for clearance of other toxic products during disease conditions.

Therefore, it is very important to measure plasma A2M and proteases in many following diseases to know the status and progress of the diseases (Khan 2016):

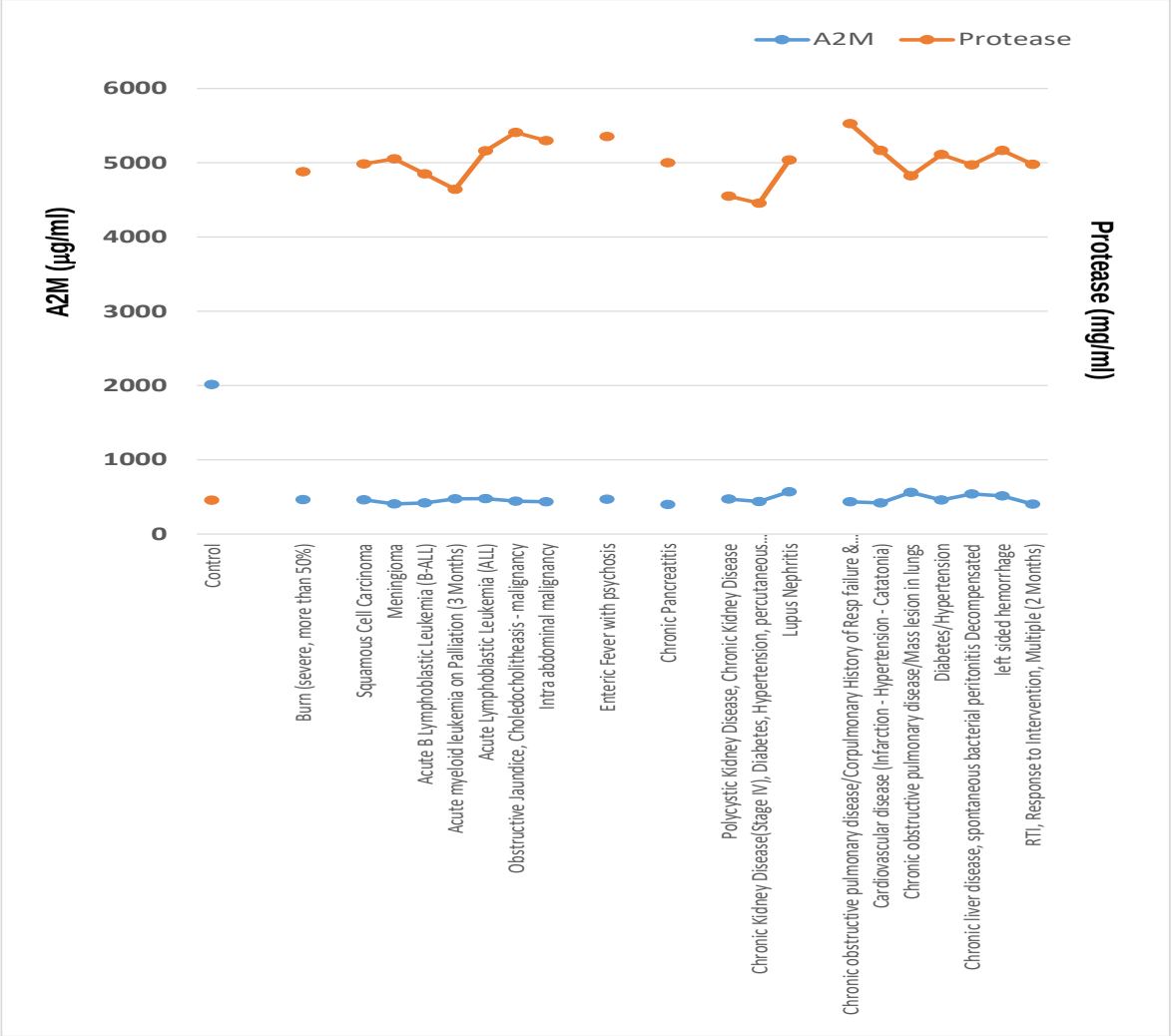
Diabetes, Hypertension, Viral infections (including HIV), Flu, Cholera, Maleria, Diarrhea, Dengue, Chicken pox, Rheumatic fever, Infection, Septicemia, Septic shock, Cerebrovascular diseases (stroke), Cardiovascular diseases : Heart attack, Any clot formation and clotting disorders, Cancers - any type, Alzheimer's disease, Autoimmune diseases, Psychiatric disorders (including Autism, Schizophrenia), Genetic disorders, Kidney disorders, Joint pain etc. and also in any disease where proteases are involved.

In a clinical study, we have shown that, in each patient, the plasma concentration of A2M, significantly went down in various types of diseases including cancer patients. In contrast, the activity of a protease (trypsin) went up from control subjects (Khan et al. 2016a; Khan et al. 2016b). (Fig. 3)

Vol. 2, No. 03; 2018

ISSN: 2581-3366

Fig 3.




To find out how NMR lives with exceptionally long life without cancer and resistant to severe adverse environment, it was discovered that the gut of the NMR is colonized by diverse, but low numbers of cultivable microbes compared with humans and mice. Interestingly the primary food of the rodents were also analyzed and found that the food (plants) are taken by these NMR are rich in **polyphenols** and related compounds, possessing anti-microbial, anti-inflammatory, anti-oxidative as well as anti-cancer activity which may contribute to their exceptionally healthy life (Debebe et al. 2014). Following this unique concept, a list of **Polyphenols** containing healthy foods were described recently (Khan 2018) which may help to prevent many disease(s) by starting elevating A2M concentration in our blood like NMR. Further investigation should be continued to find out the relationship between polyphenol containing foods and elevation of plasma A2M concentration, which may have a breakthrough concept (**A2M-ShopAnn System**-raising A2M level) to maintain our long healthy life. It could be a new

Vol. 2, No. 03; 2018

ISSN: 2581-3366

direction to investigate the unknown cause(s) of many diseases by measuring A2M and proteases. A2M could be an excellent candidate for the development of a new medicine for prevention and treatment of many diseases(s).

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Vol. 2, No. 03; 2018

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Vol. 2, No. 03; 2018

ISSN: 2581-3366

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