International Journal of Biotechnology and Biomedical Sciences
p-ISSN 2454-4582, e-ISSN 2454-7808, Volume 5, Issue 1; January-June, 2019 pp. 4-10

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Network-based Analysis of Inflammatory Biomarkers and Their Role in non-communicable Diseases

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Abstract—*Cytokines, also known as inflammatory biomarkers, play an essential role in the management of both adaptive and innate immune system. Given their significance in pro-inflammatory procedures, cytokines have been utilized for understanding the pathogenesis and as biomarkers in numerous non-communicable diseases (NCDs). Challenges are to utilize the common cytokines to better understand prediction, early detection, progression and interrelation of NCDs. Considering cytokine biomarkers as reproducibly quantifiable biological markers, studying interrelation of commonly expressed biomarkers among NCDs can implicate the therapeutic destination, observing of clinical mediations, and development of new therapeutic interventions. In the present study we mined existing databases to identify original research papers that met our pre-defined inclusion criteria of finding major cytokine biomarkers expressed in top six NCDs; Alzheimer's diseases, asthma, chronic kidney disease (CKDs), diabetes mellitus type II, cardiovascular and cardio-renal syndrome. We further extracted common inflammatory markers, IL-6, CRP, IL-4, IL-18 and IL-17 that were expressed mutually in all NCDs. Using String online tool, we outlined a novel pathway operating among NCDs using Cytoscape. After tracing and delineating the interactions among expressed cytokines, it was seen that IL13 and IL6, along with STAT3, held the most strings in cross-connectivity as compared to CRP, IL-4, IL-18 and IL-17. It can be concluded that IL-13 and IL6 may be the influencing cytokines responsible for interrelation among NCDs considered and hence, can be the new common cytokine markers for clinical utility.*

Keywords*: Non-communicable diseases, biomarkers, cytokines, Interleukins, Cell-signaling pathways.*

Introduction

Non-communicable diseases (NCDs) are becoming the leading cause of deaths in the world responsible for 70% of the 39.5 million deaths that occurred in recent years [1].Population with all age groups, countries, regions are affected by NCDs, and becoming one of the major global challenge in the 21st century [2]. NCDs, usually chronic stage of diseases, are widely considered in six main categories: Alzheimer's disease (AD), asthma, chronic kidney disease (CKDs), diabetes mellitus type II (T2D), cardiovascular dysfunction and cardio-renal syndrome (CRS)[3]. The diseases have specific biomarker expressing at different stages of onset. The biomarkers, hence, are used for clinical utility to diagnose or prognoses the condition of the patients for referral of the specific treatment. Chronic low-grade inflammation (inflammatory molecules) is a common characteristic of all NCDs. These inflammatory markers further trigger signalling pathways and regulate a large range of cellular molecules which form abnormal cellular network leading to the onset of the diseases. Therefore, the network analysis of inflammatory biomarkers may define risk factors towards progression NCDs.

Moreover, studies on NCDs such as T2D, CVDs, CKDs and CRS show inflammatory molecules to play a significant role in the disease detection and progression. Adiponectin isa clinical screening biomarker in prediabetes and diabetic individuals [4]. Researchers also used other inflammatory biomarkers like CRP and IL6 to predict the risk factors and found that increased level of these biomarkers responsible for development of diabetes mellitus in the population [5]. However, CRP level also has been found to be related with higher mortality in CVDs population [6]. Pentraxin-3 (PTX-3) is now assuming a novel and suitable biomarker in CVD subjects which can be used in new intervention in the area of CVDs [7].

CKD has presently becoming emerging cause of morbidity world-wide. IL18 used as diagnostic biomarker for acute kidney injury (AKI) population [8] along with CRS, path physiological state that consist cardiac and renal disorder in both the organs.

Taken together, there are various inflammatory biomarkers observed with downregulation or upregulation in the CRS population [9].

Various hypotheses also highlight the importance of the inflammatory biomarkers[10]. There is still the need to define new biomarkers for NCDs as the present diagnosis do not provide precise therapeutic approach. As of many, there are various inflammatory biomarkers in the Alzheimer's disease (AD) that can be used in the treatment of AD such as histamine and IL-1β (interleukin-1 beta) which have been observed in high levels in patients[11].In addition to this, IL6 (interleukine-6) and their soluble receptor showed deregulation level in the subjects suffering from AD[12]. Allergicasthmas are one of the universal and well-characterized syndromes in NCDs. There are several inflammatory markers which raised in asthma subjects for example IL3 (interleukin-3)[13] and IL17 (interleukin-17)[14].The level of hs-CRP (high-sensitivity C-reactive protein) can be utilized to detect directly severity of asthma as well [15].

Hence, inflammatory biomarkers may be considered to manage the disease associated risk events. In most of the situation, inflammation cellular signalling path ways express a cascade of events with some molecules acting as primary expressing markers which further bring about secondary line of molecules and so on. This ultimately produces a strong inflammatory ambience disrupting the patient's homeostasis. Identification of such key inflammatory targets is critical from a translational aspect in order to treat the inflammation component of the disease that can lead to slow down or even arrest in its pathological and clinical progression.

However, present study findings demonstrate the fact of high expression of key inflammatory biomarkers and their cellular signalingmolecules having a significant role in NCDs. The new approach in this area may involve precise anti-inflammatory treatment for different NCDs. The present study, for the first time demonstrates and intercalates how all NCDs express inflammatory biomarkers. The markers are put to subjective analysis to predict a common cellular signaling pathway to show their interdependency. It is, thus observed that all NCDs express common inflammatory biomarkers with IL3 and 6 being predominant among all. This paves way of using this common pathway in NCDs to predict the risk analysis of a patient developing one NCDs and their chances of developing another.

Methodology

Motivated by the previous studies and to overcome the limitation in the selection of inflammatory gene network system, we have selected all the specific inflammatory biomarkers which are expressed in major six NCDs and also analyzed their cellular signaling pathways. The biomarkers were analyzed and a common network pathway analysis was constructed with the steps followed in figure 1.

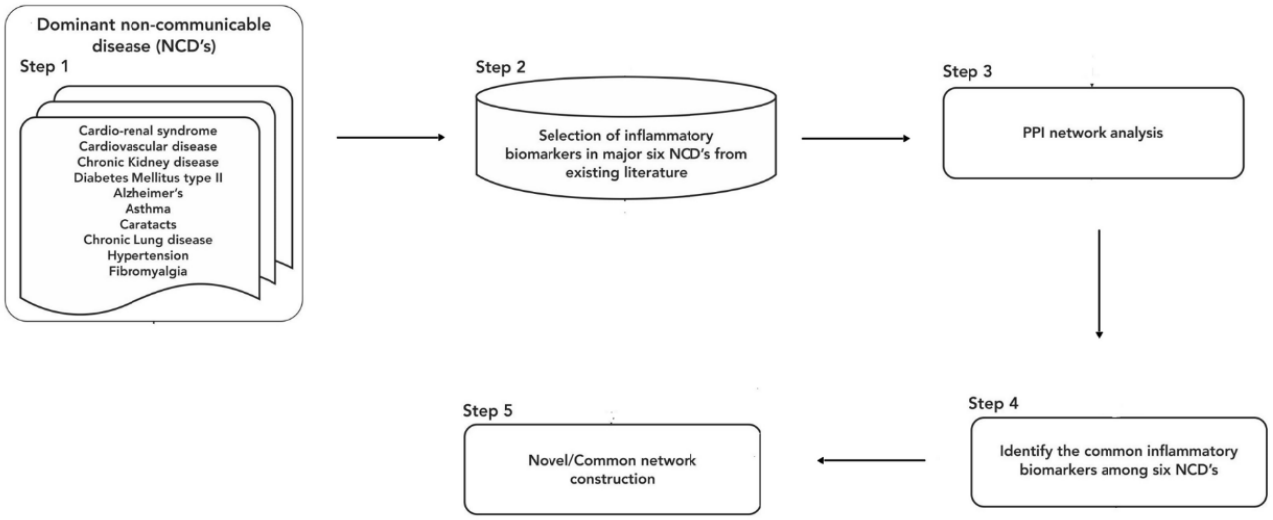

Figure 1. An overview of the work flow for the present study.

Step1: Selection of major six NCDs and their significant inflammatory markers expression among ten from existing literature; *Step 2:* PPI network analysis of inflammatory biomarkers using bioinformatics tools such as *string analysis*; *Step 3:* identification of the common inflammatory markers among the six NCDs, *Step 4:* Establishing and construction the novel interconnected cellular signaling pathway among the six NCDs using *Cytoscape 2.1 software.*

Biomarker selection

Six major NCDs were identified through existing source[16].Toidentify the specific inflammatory biomarkers expressed in NCDs, existing research sources from PubMed was collated to highlight the major expressing biomarkers. The biomarkers along with their diseases is shown in table 1.

Protein-Protein interaction (PPI) network

To know the interactive pathway of the inflammatory biomarkers, a PPI network was constructed using *STRING* online tool[17].This database produced direct or indirect network of the protein molecules showing a framework of interconnectivity among inflammatory biomarker network. It identified both experimental and predicted intercommunication based on their neighbourhood molecule, co-expression, text mining, co-directional vicinity of genes on the genome.

The integrated network analysis and pathway construction

The PPI data was downloaded from *STRING* database. The inflammatory signatures were then imported into interaction network. The PPI network analysis was visualized using *Cytoscape*2.1 software [18].In a given network, each molecule is representedas a node and interaction between the nodes known as edges.

Results

Identification of inflammatory biomarkers in NCDs

Pub Med database results revealed studies showing high expression of IL6, IL13, IL4, IL10, IL18, IL17, CRP and STAT3 biomarkers in NCDs. we have identified 15912 unique abstracts using research terms “inflammatory biomarkers” for AD, asthma, T2D, CVDs, CKDs and CRS individually. Among these, twelve publications were minedto correlate the association of inflammatory biomarkers and their cellular signaling pathway analysis.

Inflammatory biomarkers were analyzed using *STRING* analysis that revealed their interaction/co-expression with other cytokines in their cellular signaling pathways and also playing significant role in the disease prediction, identification, progression and early risk factors for the other NCDs. Moreover, novel and common biomarkers can be predicted via these signaling events, so that only specific pathway/molecules will be targeted for the medication invention and more precise treatment which is deficient somewhere in NCDs.

The study observes various biomarkers which highly express themselves in NCDs and were considered for the present study. *STRING* analysis showed co-expression of other markers and PPI network was generated with inflammatory seed genes which resulted in various interaction among nodes. The network included 68 nodes with 92 interactions having high expression of CRP, STAT3, SOCS3 and IL3.

In CRS the extended PPI network generated using the major three inflammatory seed genes CRP, IL6 and IL4 in *STRING* resulted in 25 interactions between 19 nodes. The network obtained from *STRING* was subsequently analyzed. Nodes with large degree and high degree represent the key inflammatory genes which are CRP, IL6 and IL4 that leads to the co-expression of others markers such as ALB, INS, C1S, C1R, JUN, SOCS3, CXCL8, IL1B, IL10, IL6R, IL4, STAT6, TNF, IL1B, CXCL8, CCL2. Same as CRS, PPI network were drawn for the others selected NCDs such as CVDs, CKD, T2D, AD and asthma as described in table 1. The network so constructed using key genes revealed that CRP, SOCS3, IL6 and STAT3 are common cellular signaling molecules in NCDs signaling pathways. It was observed that these signaling molecules are commonly expressed among all NCDs (figure 2).

Table 1: List of significant biomarkers and their co-expressed genes/markers in major NCDs. Bold depicts the inflammatory biomarkers expressed in all NCDs which were further considered for PPI pathway analysis.

Major six NCDs	Significantly Expressed Biomarkers	Associated (co-expressed) markers found	No. of Nodes via PPI	No. of interaction via PPI	Ref.
AD	IL-1β and **IL-6**	JUN, SOCS3, CXCL8, IL1B, IL10, IL6R, IL4	11	15	11,12
Asthma	IL-3, IL-17, **CRP**	ALB, INS, C1S, C1R	8	8	13-15
T2D	Adiponectin, **CRP**, **IL6**	ALB, INS, C1S, C1R, JUN, SOCS3, CXCL8, IL1B, IL10, IL6R, IL4	24	37	4,5

CVDs	**CRP**, PTX-3	ALB, INS, C1S, C1R	8	8	6,7
CKDs	**IL18**	IL10, IL1B, PYCARD, CASP1, IL4	6	7	8
CRS	**CRP, IL6, IL4**	ALB, INS, C1S, C1R, JUN, SOCS3, CXCL8, IL1B, IL10, IL6R, IL4, STAT6, TNF, IL1B, CXCL8, CCL2	19	25	9

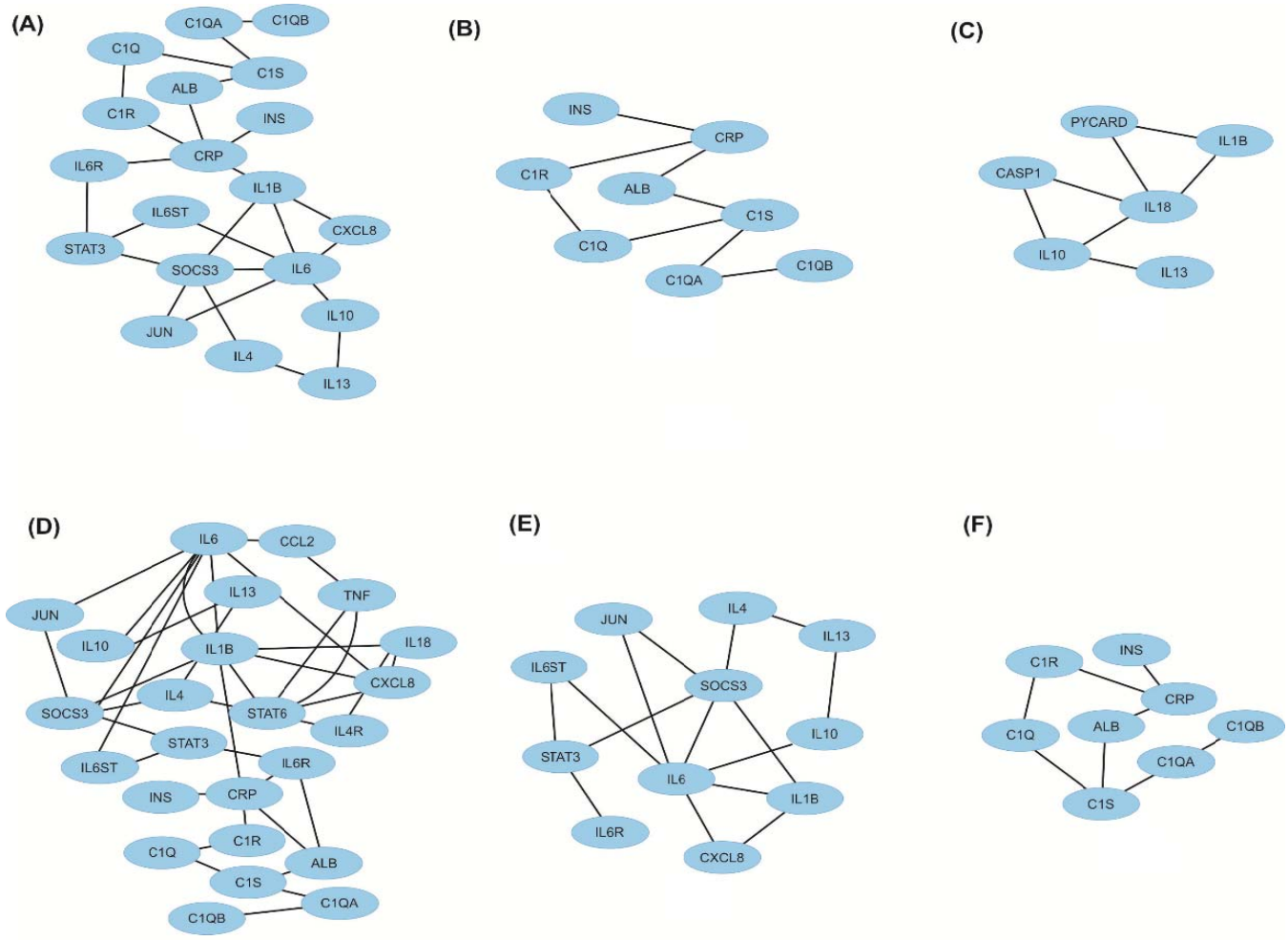

Figure 2: *PPI* cytokine network of highly expressed in the major six NCDs which represents nodes with co-expression of other proteins (A. represents CRS; B. CVDs; C. CKDs; D. DT2; E. AD; F. Asthma). Blue nodes: inflammatory interleukins, connecting lines indicates regulatory interactions between protein biomarkers.

Subjective analysis of PPI networks

Subjective analysis and observation carried out on the interactive pathways revealed that the PPI network showed interactive and common regulatory pathways operating in all NCDs. Further, a novel cellular signaling pathway was traced. Communal inflammatory genes which showed highly expressed dominant genes IL6, IL13, IL4, IL10, IL18, IL17, CRP and STAT3. The key inflammatory gens having predominant role among the NCDs consisted of eight nodes via ten interactions (figure 3a). The traced pathway showed IL13 having highest number of nodal connectivity with six nodes connected, whereas IL6 showed five node connectivity. Hence, Here, IL6 and IL13 occupied the center of the pathway, having higher expression among all NCDs, which suggest that IL6 and IL13 and their associated pathway molecules can be considered as a novel inflammatory biomarker for the NCDs (figure 3b).

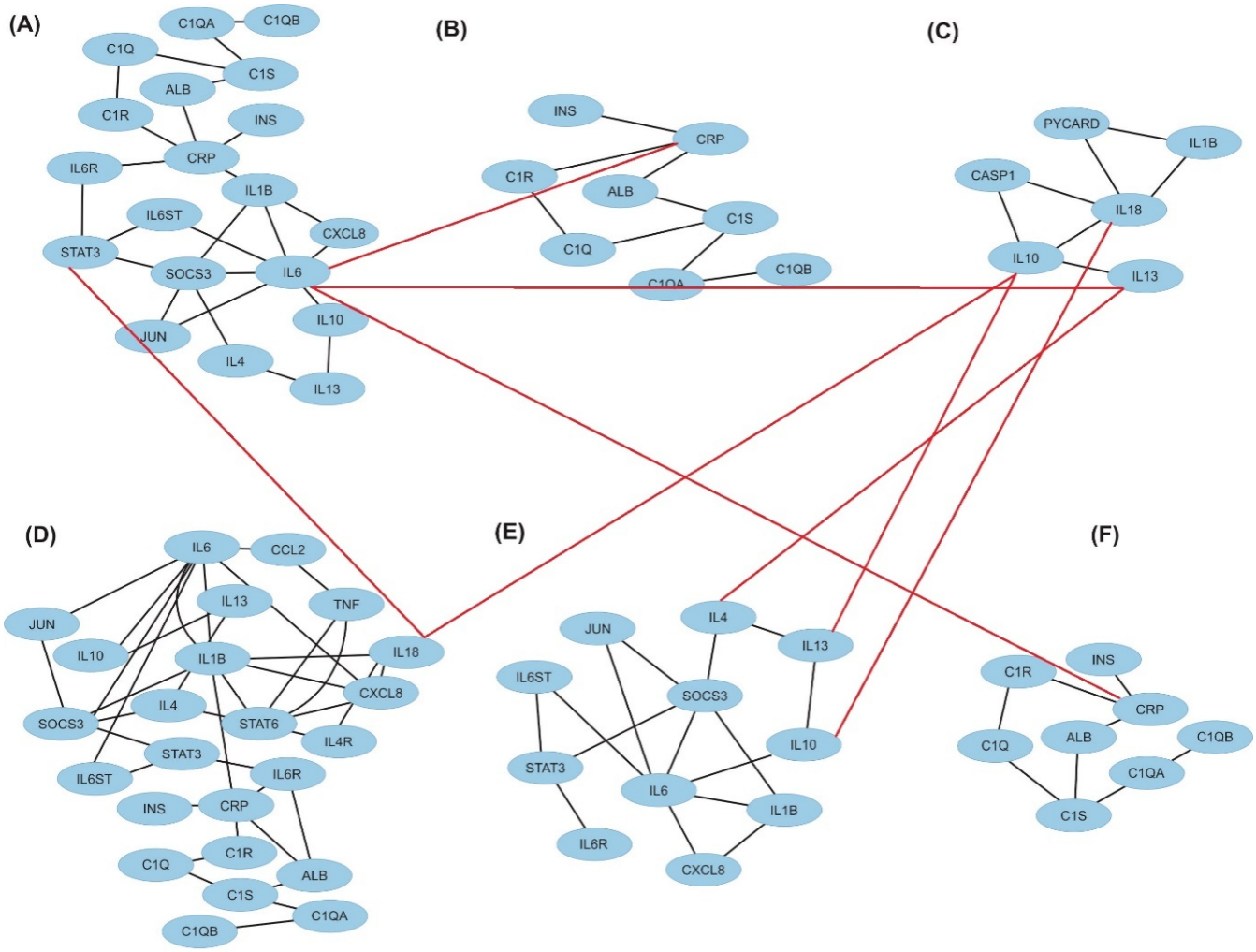

Figure 3a: Inflammatory protein co-expression. A link between two nodes represents interaction between two genes. The red lines depicts interconnection among the major six NCDs which shows common inflammatory cellular signaling molecules that might share common signaling cascade pathways.

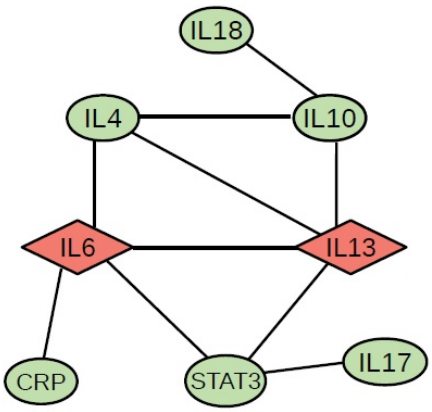

Figure 3b: Novel activated inflammatory cellular signaling pathway in six NCDs. Upregulated inflammatory cytokines; red node, Co-expressed inflammatory proteins; Green node. IL6 and IL13 represents center biomarkers which illustrate direct co-expression with IL10, CRP, STAT3 and IL4. STAT3, IL4 and IL10 forming bridge for IL17 and IL18 respectively. This inflammatory cytokine signaling cascade also predicts the expression of STAT3 as a diagnostic biomarker for six NCDs.

The current study proposes that the significant common inflammatory biomarkers among the dominant NCDs such as IL6, CRP, STAT3, IL18, IL17 and IL4and their cellular signaling events with co-expression of other genes. It has been found that IL13 and IL6 plays a significant role in among the major six NCDs.

Discussion

Drug development and treatment of NCDs has become progressively challenging and source intensive. These tasks could in part be improved by use of specific inflammatory biomarkers, suitably identifying their role in NCDs.

Inflammation is a robust feedback that is stimulated by destructive stimulus in cellular tissue injury [19, 20]. Considerable interventions have been made in better understanding of inflammatory biomarkers and cellular signaling pathways which are involved in the inflammatory responses during tissue injury in NCDs. In the present study, it was observed that IL6 and IL13 co-expressed IL6ST, STAT3, IL13 and IL6R from PPI network. The amount of IL18 may be favorable for the prediction and identification of T2D individuals having more chances of kidney dysfunction in future. As there are organ cross-talk (Heart and kidney), it also increase the possibility for CKDs and CVDs [21, 22]. Moreover urinary level of IL18 might be used as a diagnostic biomarker for the acute kidney dysfunction subjects [23].

All the listed diseases for the study are categorized in to two main categories I and II. Category-I consist of (AD, Asthma and T2D) and II (CVD, CKD and CRS). It has been found that categories I and II share common factors among the diseases and the categories too correlating with our findings that inflammatory pathways operate commonly among diseases cross-talks. It is found that IL18, IL10, IL4, IL13, IL6, CRP, IL17 and STAT3 are interconnected to each other, however it is also found that IL-6 and IL-13 inflammatory biomarkers are having direct interconnection. But it might be possible that these two independent upon each other [24]. Evidence It will always provide a significant and specific way in the prevention and management of NCDs and consider this pathway for studying and diagnosing interdependency and diseases cross-talks for the precise prediction and assigning proper drug therapy.

High expression of adiponectin in T2D have also revealed presence *i.e.* **CRP** and **IL6**. It has been found co-expression of ALB, INS, C1S, C1R, JUN, SOCS3, CXCL8, IL1B, IL10, IL6R, and IL4. On the other hand, **CRP**, PTX-3 is significantly high in CVD dysfunction subjects along with ALB, INS, C1S and C1R signaling molecules. This is consistent with our studies that if the markers of T2D are studied they might help us analyze the risk factor of T2D for AD, CVD or CKD. The same concept of studying disease cross-talk can also be applied to study organ-crosstalk among all NCDs.

Furthermore, **IL18** is significantly observed in CKD with the co-expression of IL10, IL1B, PYCARD, CASP1 and IL4. It has been observed that **CRP, IL6** and **IL4**, plays significant role in CR Salong with the association of ALB, INS, C1S, C1R, JUN, SOCS3, CXCL8, IL1B, IL10, IL6R, IL4, STAT6, TNF, IL1B, CXCL8 and CCL2. Hence, with the preliminary analysis of our study using subjective way of tracing the pathway, it may be observed that inflammatory marker pathway of IL13 and IL6 may constitute major contribution in diseases-organ crosstalk.

The current study has certain limitations. Firstly, this is a secondary study on inflammatory biomarkers in major NCDs and common markers among the same. Secondly the study only used bioinformatics tools, which means it needs experimental data for the validation of current findings in further studies.

Conclusion

There is a need for new inflammatory biomarkers that can aid in therapeutic decision making and add information about screening, diagnosis, risk stratification, and monitoring of the response to therapy in NCDs. The future lies with the examination of numerous biomarkers and their cellular signaling pathways that might be used in the development of novel diagnostic and prognostic therapeutic interventions in NCDs.

Funding

The present study was not supported by any funding

Conflict of interest statement

The authors report no declaration of interest.

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