Insecticidal Exposure may be the Cause of Progression of Immune Disorders and Inflammatory Diseases through Dys-regulation of Cytokines

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ABSTRACT

Industrial revolution has dangerously increased the load of environmental or environmentally induced diseases. Recently, the insecticides (a class of pesticides killing various insects) have become one of the most important environmental pollutants. This Review discusses the association of insecticides, cytokines dys-regulation and the development of immuno-regulatory disorders and chronic inflammatory conditions. In spite of abundant reviewing and reporting of various immuno-inflammatory diseases individually, efforts lack to understand the central pathological mechanism underlying these diseases collectively. The cytokines are extensively involved in inter-cellular communications; they are regarded attractive markers for risk stratification or patient prognosis. In this Review, an understanding has been generated about the role of various receptors, genes and signaling pathways etc. in the triangular link of cytokines, immuno-inflammatory diseases and insecticides. Such role, in a number of instances, had been those of (1) a receptor tyrosine kinase designated as c-kit or SCF receptor and (2) NFκB, the most excellently described transcription factor. The conclusion of the Review is that insecticides are linked with increasing susceptibility to various diseases especially any type of chronic inflammation through cytokine dys-regulation. Moreover, insecticidal toxicity can cause deviations in certain parameters of diagnosis. So insecticide applicators must be stressed to ensure only recommended doses and formulations of insecticides if really necessary.

Key words: Signaling pathways, pathogenesis, NFκB, laboratory tests, pesticide, diagnosis.

INTRODUCTION

Among all creatures, only humans can, scientifically, manage self-generated challenges by building additional cultural functions, e.g., cardiologists can generate knowledge about how to medicate a heart patient. However, we may not instantly be able to determine accurately some factor(s) involved in a disease.1 Frequently, such unknown factor is linked to artificial manoeuvring of agricultural activities, culminating in fatal and non-fatal medical problems.2 In recent times, insecticides have become major pollutants of industrial revolution, which disturb our external environment and also manipulate the microenvironment. Our focus in this Review is towards investigating the increased incidence of conditions with cytokine dys-regulation during last three decades; the same is the period of intensive farming with frequent use of insecticides.3

Acute phase response follows any tissue damage; macrophages and monocytes discharge cytokines. Systemic responses to cytokines include homeostatic (pyrexia etc.) and other (hematopoietic/metabolic/biochemical) changes; any dys-regulation in them causes disease. Acute phase response (initiated by same/similar endotoxic challenge) might be changed at multiple levels leading to various outcomes/conditions. Susceptibility to infectious diseases depends on the genetic basis for cytokines, but cytokine environment is also important factor to their functioning.4 Evidences of the involvement of various insecticides in...
many immune defects suggest insecticide exposure and immune defects to be linked in a broader sense.[5,6]

**Role of insecticidal toxicity in diseases:** Extensive use of insecticides in various industries, household and medicine has caused alterations in animals e.g., endotoxin-induced macrophage stimulation via restricting nuclear factor-kappa-B (NFκB) stimulation and de-oxiribonucleic acid (DNA) damage.[6,7] Links of insecticides exposures had been documented with a number of conditions e.g., disturbed immunity, infectious/inflammatory lesions of the kidneys, disturbed glucose homeostasis, diabetes, asthma, acute pancreatitis, cancers and behavioral alterations through disturbance of cholesterol mechanism.[3,5,9,10,11,12,13]

**Roles of cytokines in diseases:**
The cytokines in almost all animals especially mammals (including humans) are similar except for few additional cytokines in ruminants.[14] Proinflammatory cytokines (especially interferon (IFN)-γ) evoke microbicidal capacity of macrophages (Fig. 1).

Almost every nucleated cell can effectively make most cytokines. Concentrations of protein hormones are measured in picomolar (10^-12) concentrations changing about thousand times only. Contrarily, interleukin (IL)-4 and IL-13 (involved in CD4+ Th2 response) are responsible for immune response (Fig. 1) to parasites, allergy, wound healing, and tissue remodeling.[15] Th2 helper cells assist B cells by elaborating cytokines like dengue and human immunodeficiency virus (HIV).[16] Cytokines from adipose cells develop insulin resistance via NFκB pathway in type-2 diabetes mellitus (T2DM), obesity, high fat diet, hyperglycemia, protein kinase-C (PKC) activation and oxidative stress.[17]

Precise mechanism of cytokine dys-regulation is poorly understood. Specific cytokines may have the principal role in certain disease e.g., IL-13 is considered both necessary and sufficient for asthma. A single cytokine can express specific incorporated cellular reactions. Organized groups of cytokines track in packs and shape intricate cytokine networks. Changes in structure of cytokine genes confer extensive diversity in populations and are pertinent to be reasons of sickness. The most important proteins involved in signalling of cytokines are signal transducer and activator of transcription (STAT). Lack of STAT-6 protects from all pulmonary effects of IL-13. STAT-6 is also expressed by many immune cells.[18]

**Agriculture related diseases and cytokines:**
There is lack of responsibility during application of various chemicals in underdeveloped countries. Changed cytokines’ levels with insecticidal exposure and in many human diseases have been reported.[3] The scholastic investigations and applied community health are not properly aligned due to little investment in fighting animal diseases and zoonosis.[19] Modifications in cytokines in various tissues of animals and humans of both sexes through insecticide toxicity might have produced such diseases e.g., chlordane decreases proinflammatory cytokine IL-1β and permethrin reduces anti-inflammatory cytokine IL-10 in fetal cord serum.[20,21] Objectives of the present Review include:

- Consider possibility of the cytokines as cause of various immuno-inflammatory diseases,
- Gather evidences of diseases of being related to both insecticides and cytokines and
- Determine new insights in cytokine- and insecticide-related diseases/disorders

**Identify and classifications of Cytokines:**
Cytokines have low molecular weight and are pleiotropic signaling glyco-proteins. They are involved extensively in inter-cellular communications, including earliest immune and inflammatory reactions to noxious stimuli and in the later adaptive (specific) immune responses to microbes. They are directed by a particular gene, but can generate numerous distinct and apparently discrete phenotypic effects. Controversies subsist about differentiation of cytokines and hormones, but some features have been defined.

Concentrations of protein hormones are measured in nanomolar (10^-9); their level can change within 1/10th to 10 times only. Contrarily, cytokines are measured in picomolar (10^-12) concentrations changing about thousand fold in disease. Almost every nucleated cell can effectively make most cytokines. Contrarily, certain distinct glandular structures can only produce typical hormones.[22] There is no unanimous classification of cytokines. Both functional and structural groups of cytokines have been explained below to help readers understand this Review easily.

**Functional Types of Cytokines:**
Seven well-known groups of cytokines on the basis of functions have been discussed.[23]

**Interleukins**
ILs include numerous related proteins, formed by Th lymphocytes (particularly Th1 cells), cytotoxic T lymphocytes (TC cells) and natural killer (NK) cells.[24] ILs intercommunicate WBCs, administer immune responses,
Induce growth of lymphoid and primitive hematopoietic stem cells and instruct other cells to divide and differentiate.[25]

**Lymphokines**

T-lymphocytes secrete many cytokines grouped together as lymphokines, which play regulatory function in immune disturbances and coordinate inflammation. In a specific disease, disturbance in one subset of T-cells or the other may explain the disease mechanism.[26,27] Immature Th subset (Th0) produces lymphokines related to both Th. Th1 and Th2 cytokines inhibit functions of each other. Specific transcription factors regulate Th cells: T-bet for Th1 cells, GATA3 for Th2 cells, Retinoic acid orphan receptor-γ (RORγt) for Th17 cells, and FOXP3 for Tregs.[27]

Th1 cells produce IL-2, IFN-γ and TNF-α, which all resist intracellular pathogens, promote cell mediated immunity and produce opsonizing antibodies; many activated macrophages may contribute in this immunological function.[27] Th1 cells are the principal CD4+ T-cells, and Tc1 cells the principal CD8+ T-cells presented in COPD-lungs.[28] IFN-γ is the leading cytokine formed by Th1 and Tc1 cells and is important in COPD-inflammation by inducing chemokines, but its level is reduced in any type of asthma except very severe disease and acute exacerbations.[26] The cytokines produced by Th2 cells are involved in various immunological functions (Table 1).

GATA transcription factors have unique skill to attach the DNA sequence “GATA”. GATA-3 participates in all stages of T-cell growth (from common lymphoid progenitor to Th2), and also plays roles beyond immune system.[3] Th17 cells differentiate under the influence of TGF-β, IL-6, IL-21 and IL-23 by Th17-motivating cytokines.[27]

**Table 1: Th2 Cytokines and their functions**

<table>
<thead>
<tr>
<th>S #</th>
<th>Cytokine</th>
<th>Function(s)/disease(s)</th>
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<tbody>
<tr>
<td>1</td>
<td>IL-4</td>
<td>Delayed-type hypersensitivity, IgE Production, Alterations of pulmonary air-spaces,[29] Tc cell activation, Eosinophil-mediated inflammation[27]</td>
</tr>
<tr>
<td>2</td>
<td>IL-5</td>
<td>Supports Th1 and Th17 responses.[29]</td>
</tr>
<tr>
<td>3</td>
<td>IL-6</td>
<td>Inflammation, allergy and tumor immunity Increased IL-9 level provokes asthma[30]</td>
</tr>
<tr>
<td>4</td>
<td>IL-9</td>
<td>Inhibit Th1 cytokine synthesis, Inhibit antigen-presenting cells,[29] Production of IgE, Morphological alterations of pulmonary air-spaces, Pleiotropic in action Asthma and allergy,[29]</td>
</tr>
<tr>
<td>5</td>
<td>IL-10</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>IL-13</td>
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**Proinflammatory/inflammatory cytokines:**

Their up-regulation triggers development of several inflammatory diseases. Several infectious diseases can stimulate upregulation of inflammatory cytokines. Acute inflammatory cytokines include IL-1, TNF-α, IL-6, IL-11, IL-8 and other chemokines, granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage (GM)-CSF. In vitro studies on human keratinocytes revealed the insecticide monochlorophos to significantly increase nitric oxide, lactate dehydrogenase, malondialdehyde, nuclear changes, pro-inflammatory cytokines (TNF-α, IL-6 and IL-8) and reactive oxygen species generation.[31]

Chronic inflammatory cytokines are grouped as: humoral (IL-4, IL-5, IL-7 and IL-13) and cellular (IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12, IFNs, TGF-β, and TNF-β). Under influence of IL-12, NK cells, macrophages and T cells are motivated to form IFN-γ. IL-12 is essential to stimulate inflammatory cytokines of Th1 type which induce IFN-γ in immunity.[4] IL-6 coordinates other cytokines and creates an association between non-specific and adaptive immune systems. IL-6 is among causative factors in insulin resistance, T2DM and pulmonary damage in allergic asthma.[18] The level of IL-6 and IFN-γ in the bronchi after experimental viral infection had been lowered in progeny mice receiving insecticide methamidophos.[32]

**Growth Factors (GFs):**

Produced by leukocytes and structural cells, GFs promote continued cellular existence. GFs cause morphological modifications in airways; transforming GF-beta (TGF-β) and fibroblast GFs (FGFs) motivate fibroblast propagation (fibrosis). Other GFs include: regulatory T cells (Tregs), TGF-α, GM-CSF, stem cell factor (SCF)/c-kit-ligand/steel factor, neurotrophins, EGF and vascular endothelial growth factor (VEGF). Association amid antiviral non-specific immunity and regulatory signals of the cell growth has been described e.g., lack of epithelial growth factor receptor (EGFR) or use of EGFR results in more viral replication and impairment of antiviral genes.[33] Unusual expression or utility of SCF/KL had been reported in numerous diseases. Insecticides (e.g., pyrifluquinazon) perturb c-Kit pathways via PI3-kinase/Akt signalling pathway, the protein-rich tyrosine kinase-2 (Pyk2) and the c-Myc.[34] The level of IL-6 and IFN-γ in the bronchi after experimental viral infection had been lowered in progeny mice receiving insecticide methamidophos.[32]

**Chemokines** (Chemoattractant/Chemotactic Cytokines):

They are chemotactic for inflammatory cells and have diverse target cell specificity. Inflammatory cells themselves or lung parenchymal cells can produce chemokines. Chemokines may be either homeostatic, or inflammatory, or both. They play important roles during zygote development, embryogenesis, lymphopoiesis, myelopoiesis, hematopoiesis and cardiac and musculoskeletal development.[35] Many diseases emerge through un-controlled chemokines and chemokine receptors.[35] Any acute injury or infection triggers WBCs to the site, for which the role of chemokine (e.g., IL-8 and CXCR) is necessary (Fig. 2). Exposures to organochlorine insecticides altered these leukocyte-related immune functions through inhibition of IL-8 and CCL5 in monkeys.[36]
Chemokines have four families.

1. CC family of chemokine has CCR2 (CC chemokine receptor type 2), CCR3, CCR4, CCR8 and CCR5 as important members. The monocytes and T-lymphocytes stimulate CCR2 by chemokine (C-C motif) ligand 2 (CCL2/monocyte chemotactic protein-1). Principally, the eosinophils perform expression of CCR3, involved in chemotactic reaction to many chemokines.[36] Intravascular adherence and transmigration of neutrophils are guided by CC motif chemokine CCL3 and canonical neutrophil attractants.[37] Activation of CCR5 occurs by CCL3, CCL4 and CCL5; all of these and CCL2 are up-regulated in COPD patients. The expression of CCR5 is raised in airways of asthma patients and in COPD patients, suggesting its role in recruiting T lymphocytes, macrophages and eosinophils.[38] CCR5 and CXCR4 had been especially regarded essential to serve coreceptors during HIV entry; HIV established in vaginal and rectal mucosa had been principally reported to be CCR5-dependent.[35]

2. CX family of chemokine: The expressins of CXCR2 (and CXCR1) occurs mostly by neutrophils and occasionally by eosinophils, mast cells, T lymphocytes, basophils, endothelial, hepatic, renal and neuronal cells. The CXCR2 receptors get activated by seven morphologically similar chemokines (CXCL1, CXCL2, CXCL3, CXCL6, and CXCL8).[39] CXCL1 and CXCL8 concentrations are strikingly raised in induced sputum of patients with COPD and asthma exacerbations and draw a parallel with the higher ratio of neutrophils.[36] CXCL8 and CXCL5 show additional rise during exacerbations of COPD; CXCL8 is also increased in the airways and sputum with severe asthma.[26] The CXCR3 is highly expressed by T cells in the peripheral airways of COPD sufferers.[23] CXCR3 is stimulated by CXCL9, CXCL10, and CXCL11, they all are provoked by IFN-γ and are related to COPD severity.[39] CXCR4 receptors are expressed on Th2 cells and stimulated by CXCL12; the homeostasis depends mainly on rigid control of these cytokines.[24] CX chemokine ligand-4 (CXCL4) synchronizes communication of platelets with monocytes in acute inflammation and when CC chemokine becomes available, monocytes attack the inflamed site.[39]

3. XC family of chemokines includes lymphotactins i.e., XCL1 (lymphotactin-α) and XCL2 (lymphotactin-β). They engage NK and T cells, have only one disulfide bond and heterogenesity in structure.[40]

4. CX3 family of chemokine has CX3CL1 or CDF (chemical domain of fractalkine) as the exclusive member formed by airway epithelium after activation with IFN-γ, TNF-α and IL-1β.[40]

Anti-inflammatory cytokines

They depressingly adjust inflammatory reaction e.g., IL-12 stimulates Th1 cells to discharge IFN-γ, which may repress Th2 cytokines and allergic inflammation. TGF-β slows down CD4+ T cells, thus helping immuno-modulation. IL-10 is formed by Tregs and macrophages; it reduces the creation of several proteins (MMP-9 etc.) and numerous pro-inflammatory cytokines.[27]

TNF family

TNF is key activator of inflammation. TNF-α is secreted by many cells (e.g., macrophages, mast cells, T cells, airway smooth muscle cells and epithelial cells). TNF is highly poisonous. Almost every animal cell exhibits receptors for TNF.[25] TNFR-1 is the major cell surface receptor for TNF on hepatocytes. Activation of death domain in TNFR1 causes apoptosis; other pathways can be defending; they may activate NFkB, protein kinase activated by mitogen, or STAT pathways. NFkB and STAT pathways are disturbed with insecticide toxicity.[34]

Macrophage Migration Inhibitory Factor (MIF):

MIF is an essential effector molecule of immunity and neuro-endocrine alignment. MIF stimulates pleiotropic tasks in inflammation, malignancies/cancers, metabolic and endocrine activities. MIF is also expressed by epithelial cells. Epithelial dysplasia has been reported as consistent lesion in Wistar rats exposed concurrently to ultra-violet rays and organophosphate (OP) insecticides.[41]

Structural Types of Cytokines:

The cytokines can be divided into four types on the basis of structure.[21]
IFNs:
IFN was formerly recognized as a factor which ‘interferes’ viral replication in vitro. On the basis of type-specific receptors, IFNs have three types:
i. IFN-α, IFN-β etc. are required for immunity against most viruses.
ii. IFN-γ acts like factor activating macrophages; essential against bacteria, fungi, parasites etc.
iii. IFN-λ is further divided: IFN-λ1 (IL-29), IFN-λ2 (IL-28A) and IFN-λ3 (IL-28B).

Helical Cytokines
Growth hormone (GH), erythropoietin (EPO) and prolactin (PRL) are helical cytokines. They have two receptor binding sites: site 1 (high affinity) and site 2 (low affinity); every receptor has two domains (intracellular and extracellular) joined through transmembrane helix. When the two sites on a helical cytokine get occupied by two receptor molecules, the receptor in that cytokine is activated leading to transphosphorylation of two Janus kinase-2 (JAK2) molecules. The phosphorylation of the JAK2 molecules is followed by downstream signaling. GH axis can adjust inflammatory progression according to needs, support cell cycle successions of lymphoid tissue and check apoptosis through NFkB and phosphatidylinositol 3 (P13)-kinase/Akt pathway. EPO is formed by fully-developed kidneys. EPO is induced by low blood O2 through the transcription factors. EPO increases RBCs and plays several roles exterior to bone marrow including erythropoiesis, tissue protection and restricting apoptosis. PRL, a pituitary hormone with very important responsibility in reproduction, can also perform like a cytokine concerned with the immune reactions. In its presence, immune cells propagate fast and synthesize more cytokines through several intracellular pathways (e.g., Jak2/STAT, Ras/Raf/MAPK). The life-cycle and capacity to form pathogenic autoantibodies of autoreactive B-lymphocytes is extended by PRL in systemic lupus erythematosis.

IL-2:
IL-2 shows pleiotropy; it forces the T-cells to grow, boosts up NK cells, stimulates proliferation of regulatory T cells and is involved in cell death induced through receptor activation. IL-2 may prime/sustain/develop Th1 and Th2 segregation; while it inhibits Th17 segregation but can also develop Th17 cells. Insecticides reduce the skill of well-purified human NK cells to lyse tumor cells through imbalancing NK-inhibitory (IL-4) and NK-stimulatory (IL-2, IL-12 and/or IL-10) ILs. Some insecticides bring out concordant signaling pathways (e.g., JAK-STAT) in the mouse models having common characteristics with some pathways detected in human Parkinson’s disease; Parkinson’s type neurodegeneration had also been reported in insecticide-exposed rural peoples.

IL-10 Subfamilies of Cytokines:
They include IL-10, also called cytokine synthesis inhibitory factor (CSIF), cytokines resembling IL-10 (IL-19, IL-20, IL-22, IL-24 and IL-26) and type-III IFNs (IL-28A, IL-28B and IL-29), which less distinctly resemble IL-10. IL-10 family cytokines are critical to epithelium homeostasis; they contribute in several infectious and inflammatory diseases. TNF-α and IL-10 are involved in tuberculosis (TB). Certain single nucleotide polymorphisms (SNPs) within the promoter region of the IL-10 and TNF genes have been associated with altered levels of circulating IL-10 and TNF-α. IL-10-1082G and positive relationship of IL-10-1082GG and IL-6-174GC promoter region polymorphism with coronary artery disease (CAD) in Pakistani population suggests that upset in the Th1/Th2 balance may manipulate the vulnerability of CAD. Insecticides (e.g., permethrin) had been negatively linked with IL-10, indicating their role in susceptibility to TB and CAD.

Diagnostic tests versus diseases, Cytokines and Insecticides:
Diagnostic laboratory tests can assess the inflammatory response, various cytokines and cytokine-related diseases; insecticide toxicity may doubt their results. In the following paragraphs, hemoglobin (Hb), erythrocyte sedimentation rate (ESR), c-reactive proteins (CRP), leukocytes, pancreatic functions, liver function tests (LFTs), kidney function tests (KFTs), blood glucose and various hormones have been discussed in the respective scenario.

Hb:
Cytokines in chronic inflammatory conditions mediate immune processes leading to hypoferremia leaving microbes without iron, but by some faults iron-restricted erythropoiesis and anemia may result. In mature non-diabetic peoples, increased danger of diabetes and more robustly the danger of heart disease and mortality had been linked with glycated Hb (HbA1c) relative to fasting glucose. Low Hb concentrations had been linked with disease severity of rheumatoid arthritis (RA) etc. The anemia development, decreased Hb and mean corpuscular Hb concentrations had also been suggested in insecticide-toxicity studies.

ESR, CRP and Red Cell Distribution Width (RDW):
Combination of ESR and CRP readings at 24 hours forecasts RA susceptibility and severe acute pancreatitis (SAP) accurately; TB and diabetes are also linked with them. Standard range of ESR had been reported in most dengue patients at any stage, differentiating it from bacterial diseases. ESR is a vital indicator of active ulcerative colitis (UC) and RDW is a sensitive and specific indicator for verifying active Crohn’s disease (CD). High RDW is an autonomous feature associated with fatal heart failure in aged persons. Reduced RDW is noted in multiple sclerosis, melancholy and hypertension.
CRP levels were raised 3-5 fold, while RDW lowered in insecticide sprayers. \[^{57,58}\] ESR increased following both acute and sub-acute exposure of chlorpyrifos insecticide in fish. \[^{59}\]

**Leukocytes:**
Basic module of innate immunity is neutrophils’ relocation into inflamed tissues; blood neutrophils undergo polarized movement from first to last endothelial cells (ECs) coating the venular lumen in a luminal-to-abluminal route. During this transendothelial migration, enzymes convert lysophosphatidylcholine (LPC) to lysophosphatic acid. Neuropathy target esterase (NTE) hydrolyzes LPC in vitro. \[^{60}\] NTE is a secondary target of some insecticides; insecticides damage the leukocyte functioning by attacking NTE and favouring cytotoxic accumulation of LPC to rupture ECs. \[^{61}\]

**Proteins:**
Insecticides may attack to albumin, lipoproteins and to alpha-1-acid glycoproteins (AGP). AGP determinations are considered diagnostic for inflammatory bowel disease (IBD) and CD. \[^{12}\] The mitochondrial translocator protein (TSPO) has enormous attraction to bind with drugs and cholesterol and is highly expressed in colon cancers and also in initial inflammation in IBD. The mixture of TSPO, voltage-dependent anion channel and adenine nucleotide transporter binds various pollutants including insecticides. \[^{62}\] The evaluation of IBD in dogs through canine IBD activity index (CIBDAI) is insufficient to predict disease status, so combination of CIBDAI, histologic score, and serum albumin level is used for sufficient curative strategies. Albumin is an essential parameter for dietary management of chronic pancreatitis and scrutinizing diabetic pathophysiology. \[^{63}\] Globulin is also involved in some diseases e.g., group-specific component globulin, Ge-globulin is a prognostic and treatment marker in SAP. \[^{21,64}\] Deviation in serum albumin and globulin concentrations and their structural and functional features resulting from insecticidal treatment/exposure may lead to misdiagnosis and/or mismanagement and/or exaggeration of these conditions. \[^{6}\] Insecticides slow down calcium recruitment in G-protein-coupled receptor (GPCR) leading to GPCR desensitization and internalization through stimulation of conventional PKC. \[^{65}\]

**Blood Glucose:**
Acute hyperglycemia exaggerates hyperinsulinemia and upregulates circulating cytokines. \[^{66}\] Impaired glucose tolerance (IGT) in sepsis is linked with alterations in plasma glucagon and cortisol and inflammatory cytokines (IL-6 and TNF-α); it is because IGT builds up acute hyperglycemia through glucose infusion. \[^{61}\] Expressions of all glucose-6-phosphate isomerase-derived indicators in RA are elevated. Insecticides break glucose homeostasis, stimulate momentous pancreatic injury and cause oxidative damage to pancreas. \[^{10}\] The suggested mechanism involves stimulation of adrenergic receptors (AR); it includes hyperlactacidemia through β-AR and lactate consumption and hyperglycemia through α-AR. \[^{66}\]

**LFTs:**
Hepatic dysfunction had been described in children with serologically confirmed dengue infection; its severity in dengue hemorrhagic fever and DSS (dengue shock syndrome) patients is more than that in dengue fever patients. Similarly, liver dysfunction is extremely frequent in all types of dengue infectivity in adults, with aspartate transaminase (AST) ascending drastically higher than alanine transaminase (ALT). Serum levels of bilirubin, ALT and alkaline phosphatase are significantly raised in long-suffering peoples with DSS, haemorrhage and sequential infection. \[^{54}\] The insecticides also lead to elevated transaminases. \[^{6}\]

T2DM also deviates the LFTs, including reciprocal alterations in two endoplasmic reticulum enzymes (hexose-6-phosphate dehydrogenase and isocitrate dehydrogenase); these enzymes produce NADPH and determine the directional activity of hepatic 11β-hydroxysteroid dehydrogenase (11β-HSD1). \[^{67}\] Several insecticides utilize carbonyl moieties in their mechanism of activity; carbonyl reductase in liver microsomes is chiefly credited to 11β-HSD1. \[^{68}\] Subtle chronic rises of transaminases regularly had been considered a sign of causal insulin resistance, it is notably true for ALT and especially in Asian populations. \[^{6}\] The link of insecticides toxicity (e.g., imidacloprid) and life-threatening multi-organ dysfunctions may create diagnostic difficulties in such patients after contact with insecticides. \[^{69}\]

**Pancreatic Function Tests:**
The initial stage acute pancreatitis is detected more clearly by IL-8 serum levels than those of CRP and TNF-α to differentiate it from high-risk patients with severe disease. \[^{25,54}\] Proton pump inhibitors (PPIs) can recover glucose dysregulation and stimulate pancreatic beta-cell neogenesis in T2DM. PPIs undergo metabolism through cytochrome P450 system and insecticides decrease cytochrome P450 17α-hydroxysteroid dehydrogenase (P450 17α). \[^{3,70}\]

**KFTs:**
T2DM patients are under greater threat to chronic kidney problems, which in turn cause heart related deaths. Balanced activation of peroxisome proliferator-activated receptors (PPARs) i.e., PPARδ and PPARγ in diabetes can improve insulin sensitivity. \[^{71}\] A 3-hydroxy, 3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor atorvastatin is included in sub-network of PPAR. Some insecticides prospectively modulate the course of PPAR signaling and HMG-CoA reductase. \[^{5}\] Tamm-Horsfall protein (THP) had been assumed to perform regulatory function of renal and systemic immunity manipulating cytokine excretion. \[^{72}\] Insecticides when acting on renal tubules diminish secretion of THP. \[^{73}\]
**Cytokines**: The rise of cortisol concentration in T2DM is unaccompanied by adrenocorticotropic hormone fall, which verifies essential fault in hypothalamic-pituitary-adrenal (HPA) axis. Cytokines influence the neuroendocrine processes in all stages of reproduction; elevated plasma/serum LH in insecticide exposed male subjects occur due to normal negative feedback in response to the decreased testosterone hormone of the HPA.[74] Impaired insulin and glucose metabolisms had been connected with many diseases (e.g., CD, TB and RA), in addition to diabetes.[15] Insecticides reduce glucose oxidase activity and deteriorate glucose tolerance in animals and also disturb neurotransmitter or ion channel systems.[166] Vitamin D upregulates mitogen-activated protein kinase phosphatase-1, reduces lipopolysaccharide (LPS)-induced p38 stimulation and prevents monocytes/macrophages from synthesis of several cytokines.[60] Insecticides had been linked with vitamin D deficiency in humans and thin avian egg shells, strongly connected to Ca+2 metabolisms (controlled by estradiol and vitamin D).[75]

**How Cytokines dys-regulate?**

Inflammation sets in when innate immune cells perceive an insult and cytokines are stimulated. Fig. 3 depicts normal functioning of cytokines. Supervision of the inflammatory process involves pattern recognition receptors (PRRs); nearly all PRRs take action to pathogen-associated molecular patterns through various transcription factors e.g., NfκB.[20] NfκB gets activated in any oxidative stress. Differences within cytokine genes have been associated with diversity within individuals in their immune response and in resistance to multiple pathogens. [76]

![Figure 3: Normal functioning of cytokines](image)

During cellular responses of acute inflammation, chemokines are expressed as attached to proteoglycans. WBCs get stimulated and receptor avidity of integrins gets enhanced, so that additional integrins attach to proper ligands on WBCs and bunch together. During vascular reactions in acute inflammation, cytokines lead to a sluggish and delayed withdrawal of endothelial cells; cytokines support appearance of selectins and integrin ligands.[17] The enhanced integrin avidity and enhanced appearance of integrin ligands at the inflammatory sites consequently lead to firm binding of WBCs to endothelium. In the presence of products of bacteria (e.g., endotoxins, LPS), activated macrophages, endothelial cells and mast cells secrete TNF and IL-1. TNF enhances endothelial thrombogenicity and stimulates neutrophils; IL-1 stimulates tissue fibroblasts leading to extra-cellular matrix (ECM) accumulation.[78] Both TNF and IL-1 can be presented at far-off sites via blood to bring the systemic acute phase reaction and can also operate as endogenous pyrogens, raising the concentration of cyclo-oxygenase to facilitate conversion of amino acid into prostaglandins (PGs). In the hypothalamus, PGs (particularly PG-2) motivate formation of neurotransmitters, which function to retune the body temperature scale at an upper point. Acute phase proteins (several hundred times than normal) are expressed by the liver in systemic inflammation.[79] The most excellently recognized acute phase proteins (i.e., CRP, fibrinogen and SAA) are up-regulated by cytokines (particularly IL-6). Fibrinogen attaches to red corpuscles leading to rouleaux formation; ESR is an easy experiment for the systemic inflammation.[80] Depending upon the stimulus, inflammatory responses can be initiated by any of PRRs (Toll-like receptors, receptor for advanced glycation end products, and scavenger receptors). Ligation of a PRR stimulates signal transduction pathways and control of various transcriptional and post-transcriptional molecules (i.e., constituents of NfκB, IFN regulator factor families and activator protein-1); pro-inflammatory target genes are adjusted encoding cytokines, enzymes, and other molecules necessary for pathogen removal.[20] During a chronic inflammation, some cytokines (especially IFN-γ, IL-12) contribute the key role. Macrophages having longest half-lives carry out most efficient phagocytosis than other cells (lymphocytes, monocytes, eosinophils, plasma cells and mast cells).[20] Macrophages get activated by various activation signals e.g., cytokines, other inflammatory mediators, ECM proteins and different microbial products.[15] In chronic inflammation, macrophages accrue and propagate instead of dying off into lymphatics. The cytokines sequester the lymphocytes in lymph-nodes so that leukocopenia follows bacterial, viral, rickettsial and protozoal diseases. Both B and T-cells can travel into inflammatory sites by means of various adhesion molecules and chemokines and engage other WBCs.[79] Balanced discharge of various cytokines is essential for recruiting macrophages in inflammatory sites. IFN-γ is capable of motivating macrophages to combine into giant cells. Macrophages present antigens to T-cells, express membranous molecules (i.e., co-stimulants) and secrete cytokines (particularly IL-12) that excite T-cell responses; again cytokines are produced (e.g., IFN-γ). The mucosal dendritic cells and mononuclear phagocytes maintain the stability of resistance to pathogens and acceptance to antigens resulting from bacterial flora and food. This stability is synchronized by both cellular communications and the discharge of cytokines. An example of dysregulated cytokines functioning has been presented in Fig. 4. Various diseases occur due to improper and persistent activation/polarization of immune cells leading to tissue damage.[15] The insecticides frequently produce reactive oxygen species which cause cellular
damage via lipid peroxidation, motivation of NFκB, mitochondrial injury and apoptosis in various (pulmonary, renal, hepatic, endocrine, neuronal) consequences.[1,6]

Further clinical and experimental investigations are needed to give details of mechanisms involved in these alterations.

**METHODS OF EVALUATIONS**

This study is a multidepartment and multiprofessional joint venture. Various studies referred in the Review are those carried out on insecticide sprayers and patients of tuberculosis, dengue, diabetes, RA, UC, CD, pancreatitis etc., with description of cytokines involvement. Various cytokines have been reviewed for related information. The inflammatory or acute phase response can also be assessed by diagnostic laboratory tests. So, Hb, ESR, CRP and leukocytes have been included to know the respective link. Acute phase responses stimulate down-regulation of albumin production and decreases total proteins and albumin-globulin ratio. So these proteins along with the LFTs, pancreatic function tests, KFTs, blood glucose and various hormones have been discussed in the respective scenario. The parameters having some link with cytokine related disease(s) and relevance to the insecticide exposure have been indicated. The selection criteria in the description had been to search in the Google Search Engine with key words of the disease name, the diagnostic laboratory tests and sorting research articles from the current decade and if not available then for older ones. The articles having linked the disease and cytokines were regarded the favourite ones for inclusion in the description. Similarly the material about insecticide poisoning in the relevance to particular disease and particular parameter of interest under review were also searched, sorted and described appropriately.

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