CHAPTER 2

Review of Literature

Asthma

2.1 Definition

Asthma is a disorder defined by its clinical, physiological, and pathological characteristics. The predominant feature of the clinical history is episodic shortness of breath, particularly at night, often accompanied by cough. Wheezing appreciated on auscultation of the chest is the most common physical finding. The main physiological feature of asthma is episodic airway obstruction characterized by expiratory airflow limitation. The dominant pathological feature is airway inflammation, sometimes associated with airway structural changes. Based on the functional consequences of airway inflammation, an operational description of asthma is (24)

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment.

The guidelines of National Asthma Education and Prevention Program (NAEPP) (1997) of heart, lung and blood institute, defined asthma as (81)

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role: in particular, mast cells, eosinophils, neutrophils (especially in sudden onset, fatal exacerbations, occupational asthma, and patients who smoke), T lymphocytes, macrophages, and epithelial cells. In susceptible individuals, this inflammation causes recurrent episodes of coughing (particularly at night or early in the morning), wheezing, breathlessness, and chest tightness. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment.
2.2 Epidemiology

Global

Asthma is ranked among the top 10 prevalent conditions and affects about 300 million people worldwide and is projected to increase to 400 million by 2025 (1). Globally asthma prevalence increases by 50% every decade (1, 2). In year 2010, the number casualties from this disease was more than 300 thousand (82). Prevalence estimates in children ranges from 3% to 38% (83) where as in adults from 2% to 12% (84).

![World map of prevalence of asthma](image)

**FIGURE 2.1:** World map of prevalence of asthma (1)

Prevalence rates for asthma around the world differ substantially. The European Community Respiratory Health Survey (ECRHS) assessed geographic variation in asthma in 140 000 adults from 22 countries. A six fold variation in prevalence of current asthma was found among the countries (85). A high prevalence (>7%) countries were Australia, New Zealand, United States, Ireland, and the United Kingdom whereas less than 4% was found in Iceland, parts of Spain, Germany, Italy, Algeria, and India. The ISAAC study provides the most extensive information on variation in childhood asthma prevalence throughout the world. The ISAAC Steering Committee reported findings for 463801 children ages 13 to 14 years in 56 countries, and 257 800 children ages 6 to 7 years in 38 countries (86). Across countries, there was an approximate 20 fold range of prevalence, with the highest rates generally in more developed countries. After an average of 7 years, ISAAC investigators surveyed 193404 children aged 6 to 7 years in 37 countries and 304679 children aged 13 to 14 years in 56 countries. The countries with the highest prevalence rates (>20%) were the Isle of Man, United Kingdom, New Zealand, Ireland, Australia, Peru, Panama, Costa Rica, the United States, and Brazil (83).
India
Asthma rates are officially low in India, although there is some recent evidence that the true prevalence is higher than previously thought. The overall prevalence of asthma is a 3% (30 million patients) among the adults over the age of 15, and median prevalence is 2.4%. However, the estimated prevalence rates vary in various parts of India and ranges from 2.4-6.4% (3). According to the conducted cross-sectional nationally representative National Family Health Survey (NFHS)-3, the overall prevalence of asthma among adult men and women in India is similar with 1,696 and 1,627 per 100,000 respectively (4). The number of men and women with asthma increases steadily with age (Fig. 2.2). Prevalence of asthma is higher in rural areas (1,719 per 100,000 for women and 1,799 per 100,000 for men) than for urban areas and that it is more common among women than men. Asthma among men is more prevalent in the lower wealth quintiles than among the higher wealth quintiles. Moreover, prevalence is highest among those with less than five years of schooling (2,283 per 100,000 among women and 2,640 among men per 100,000), and among those with no education (1,914 among women per 100,000 and 2,440 among men per 100,000) (4).

**FIGURE 2.2:** Asthma prevalence among men and women in India by broad age groups and according to residence.

2.3 Economic burden of asthma
Economic burden of asthma is very widespread in the reality. The economic cost of asthma is considerable both in terms of direct medical costs (such as hospital admissions and cost of pharmaceuticals) and indirect medical costs (such as time lost from work and premature death). Globally, the economic costs associated with asthma exceed those of tuberculosis and HIV/AIDS combined. Developed economies can expect to spend 1 to 2% of their health-care budget on asthma (1). The estimated total cost of treatment of chronic and acute cases of asthma according to current practices for 1996 to 2016 is presented in Fig. 2.3 (87).
2.4 Etiology

The complete causes of asthma are unknown. There are two categories of asthma: allergic or extrinsic and idiosyncratic or intrinsic. Allergic asthma is a result of an antigen\antibody reaction on mast cells in the respiratory tract. This reaction causes the release of inflammatory mediators from mast cells, which elicit the clinical response associated with an asthma attack. Idiosyncratic asthma is a result of neurological imbalances in the autonomic nervous system (ANS) in which the alpha and beta-adrenergic as well as the cholinergic sites of the ANS are not properly coordinated. Onset of asthma between the ages of 5 to 15 years usually indicates asthma with an allergic basis (6, 81). Exposure to environmental allergens can trigger asthma symptoms. Among the most common allergens are microscopic droppings of dust mites and cockroaches, airborne pollens and molds, plants and plant proteins, enzymes, and pet dander (minute scales of hair, feathers, or skin). Exposure to a variety of occupational irritants (e.g., vapors, dust, gases, fumes, tobacco smoke, air pollution) also can worsen or cause asthma. Certain medications may trigger asthma symptoms. These include beta-blockers, used to treat high blood pressure, heart disease, and glaucoma (in eye drops). About 5% to 20% of adults with asthma have attacks triggered by sensitivities or allergies to medications such as aspirin, ibuprofen,
indomethacin, and naproxen. Others react to sulfites (chemicals commonly used to preserve foods such as tuna, salads, dried apples and raisins, and beverages such as lemon juice, grape juice, and wine). Other factors that may contribute to asthma or worsen symptoms include sinus infections, gastro esophageal reflux disease (GERD), pregnancy, menstruation, and even the time of day. Asthma also can be induced by exercise or cold air (6, 81).

2.5 Classification of asthma

**TABLE 2.1:** Classification of asthma based on National Asthma Education and Prevention Program, USA (81)

<table>
<thead>
<tr>
<th>Classification*</th>
<th>Symptoms</th>
<th>Night symptoms</th>
<th>Lung function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1: mild intermittent asthma</td>
<td>Symptoms occurring twice a week or less</td>
<td>Symptoms occurring no more than twice a month</td>
<td>FEV1/FVC is 80% or more of predicted</td>
</tr>
<tr>
<td></td>
<td>No symptoms and normal PEF between exacerbations</td>
<td></td>
<td>PEF variability of less than 20%</td>
</tr>
<tr>
<td></td>
<td>Brief exacerbations (lasting a few hours to days) with variable intensity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 2: mild persistent asthma</td>
<td>Symptoms occurring more than twice a week</td>
<td>Symptoms occurring more than twice a month</td>
<td>FEV1/FVC is 80% or more of predicted</td>
</tr>
<tr>
<td></td>
<td>Exacerbations may affect activity</td>
<td></td>
<td>PEF variability of 20 to 30%</td>
</tr>
<tr>
<td>Step 3: moderate persistent asthma</td>
<td>Daily symptoms</td>
<td>Symptoms occurring more than once a week</td>
<td>FEV1/FVC is greater than 60% but less than 80% of predicted</td>
</tr>
<tr>
<td></td>
<td>Daily use of inhaled short-acting beta agonist</td>
<td></td>
<td>PEF variability of greater than 30%</td>
</tr>
<tr>
<td></td>
<td>Exacerbations affect activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exacerbations occur more than twice a week and may last for days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 4: severe persistent asthma</td>
<td>Continual symptoms</td>
<td>Frequent symptoms</td>
<td>FEV1/FVC is 60% or less of predicted</td>
</tr>
<tr>
<td></td>
<td>Limited physical activity</td>
<td></td>
<td>PEF variability of greater than 30%</td>
</tr>
<tr>
<td></td>
<td>Frequent exacerbations</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PEF = peak expiratory flow; FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; FEV1/FVC% = FEV1 as percentage of FVC.

*--The initial classification is based on the presence of certain clinical features before treatment. The presence of one of the features of severity is sufficient to place a patient in that category. A patient should be assigned to the most severe grade in which any feature occurs. The characteristics noted in this classification are general
Chapter 2

Review of Literature

and may overlap because asthma is highly variable. Furthermore, a patient's classification may change over time.

2.6 Symptoms
People with asthma have symptoms when the airways are narrowed (bronchospasm), swollen (inflamed), or filled with mucus. Common symptoms of asthma include (81):

- Coughing, especially at night
- Wheezing
- Shortness of breath
- Chest tightness, pain, or pressure

2.7 Pathophysiology of Asthma
The pathophysiology of asthma is characterized by variable degree of airway obstruction secondary to bronchial smooth muscle constriction, airway wall inflammation and edema, epithelial desquamation, mucous hypersecretion, bronchial hyperresponsiveness and in some case but not all, airway remodeling. Airway inflammation is characterized by an influx of eosinophils, neutrophils, lymphocytes and degranulated mast cells. Airway inflammation is considered to be primary pathologic event in asthma and the inflammatory process is the final result of a complex network of interactions between various cell lineages, its mediators and secreted substances (Fig. 2.4) (8, 13).
Chapter 2

2.8 Mechanisms in the Development of Airway Inflammation

Inflammation has a pivotal role in the pathophysiology of asthma. Airway inflammation involves an interaction of many cell types and multiple mediators with the airways that eventually results in the characteristic pathophysiologica features of the disease: bronchial inflammation and airflow limitation that result in recurrent episodes of cough, wheeze, and shortness of breath. The pattern of airway inflammation in asthma does not necessarily vary depending upon disease severity, persistence, and duration of disease however cellular profile and the response of the structural cells in asthma are quite consistent.

2.8.1 Inflammatory Cells

Many different types of inflammatory cells are involved in asthma but the precise role of each cell type is not yet certain (8, 13). It is evident that no single inflammatory cell is able to account for the complex pathophysiology of asthma, but some cells are predominant in asthmatic inflammation.

FIGURE 2.4: Pathogenesis of bronchial asthma
2.8.2 Mast cells
Mast cells release several chemical mediators such as histamine, leukotrienes, and prostaglandin which results in bronchoconstriction. In asthma mediators are released by various environmental triggers, such as allergens, and an increase in plasma osmolality as a result of increased ventilation during exercise. Mast cells also release cytokines that are linked to allergic inflammation, including interleukin (IL)-4, IL-5 and IL-13. The presence of mast cells in the airway smooth muscle has been linked to airway hyper-responsiveness in asthma (10).

2.8.3 Eosinophils
Eosinophils begins in the bone marrow and is regulated by IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor. IL-5 induces terminal differentiation of immature eosinophils (88) and mature eosinophil has dense intracellular granules that are sources of inflammatory proteins, including major basic protein, eosinophil-derived neurotoxin, peroxidase, and cationic protein. Major basic protein, can directly damage airway epithelium, intensify bronchial responsiveness, and cause degranulation of basophils and mast cells. These effects increase the severity of asthma. The eosinophil is a rich source of leukotrienes, particularly the cysteinyl leukotriene C4, which contracts airway smooth muscle, increases vascular permeability, and may recruit more eosinophils to the airway. A number of cytokines regulate the function of eosinophils and other cells in asthma. Interleukin-5 stimulates the release of eosinophils into the circulation and prolongs their survival (89).

FIGURE 2.5: The role of eosinophils in allergic inflammation
2.8.4 Neutrophils
Neutrophils are not a predominant cell type observed in the airways of patients with mild-to-moderate chronic asthma, they appear to be a more prominent cell type in airways and induced sputum of patients with more severe asthma. Also in patients who die suddenly of asthma large numbers of neutrophils are found in the airways, although this may reflect the rapid kinetics of neutrophil recruitment compared to eosinophil inflammation. Neutrophils secrete a variety of inflammatory mediators, including proteases, cytokines (e.g., tumor necrosis factor, transforming growth factor), and reactive oxygen species, which can cause airway epithelial injury and mucus hypersecretion. After activation, neutrophils release myeloperoxidase (MPO) together with other granule enzymes. MPO may then react with \( \text{H}_2\text{O}_2 \) generated during the respiratory burst together with a halide (usually \( \text{Cl}^- \)) to generate HOCl and other related compounds with wide biological activities (12).

2.8.5 Basophils
The role of basophils in asthma is uncertain; however, a small increase in basophils has been observed in the airways of asthmatic patients. Like mast cells, basophils also release histamine upon activation but they do not produce \( \text{PGD}_2 \). Further, basophils have also been found to be a rich source of IL-4 and IL-13 demonstrating both spontaneous release and response to IgE-mediated stimuli (90).

2.8.6 Lymphocyte
Lymphocytes involved in the allergic response of asthma and play a significant role in degranulation of mast cells and basophils. Lymphocytes can be categories as T-lymphocytes and B-lymphocytes. T-lymphocytes play a very important role in coordinating the inflammatory response in asthma through the release of specific patterns of cytokines, resulting in the recruitment and survival of eosinophils and in the maintenance of mast cells in the airway (13). There are two types of T-lymphocytes; helper CD4+ T cells and killer CD8+ T cells. There are four distinct subsets of CD4 T-lymphocytes, Th1, Th2, regulatory T cells (Treg), and Th17 cells can differentiate from precursor T cells at the time of antigen presentation and influence cytokine production. The differentiated Th cells are characterized by the specific sets of cytokines they release when stimulated. For many years, it was believed that asthma and other allergic diseases resulted from an imbalance between Th1 and Th2 responses in favor of a Th2 response, promoting an allergic diathesis based on Th2
cell responses. The identification of transcription factors controlling Th1 and Th2 differentiation has provided further support for a predominant role of Th2 cells in the pathogenesis of asthma, because Tbet expression is reduced in the asthmatic lung and GATA-3 is overexpressed (91, 92). However, recent discoveries and an improved understanding of the interaction between Th17 cells and regulatory T cells have shown that the simple Th1/Th2 hypothesis is not sufficient to explain asthma.

2.8.7 Th1, Th2, Th17 and Treg Lymphocytes in Asthma
Th1 is a cell-mediated immune response and is characterized by the production of IFN-γ, TNF-α, and IL-2. Th2 is humoral responses and is characterized by their secretion of IL-4, IL-5, IL-9, and IL-13. Antigen recognition by naive T cells in the presence of IL-12 favors STAT4 and Tbet activation which promote Th1 cell differentiation. Early IL-4 production favors STAT6 and GATA3 activation, which lead to Th2 cell production. Th1 cells regulate delayed-type hypersensitivity reactions, and Th2 cells are prominent in allergic reactions. Th1 and Th2 cells interact in a counterregulatory fashion; for example, IL-10 downregulates cytokine production by Th1 cells and IFN-γ inhibits the proliferation of Th2 cells. Th17 produce IL-17 and IL-22 (91, 92). These T cells are proinflammatory and have been studied primarily in mice. They express a characteristic transcription factor, retinoic acid orphan nuclear receptor (ROR-γ). In a murine model of allergic asthma, IL-17 was found to be necessary for the induction of allergic asthma. In healthy human subjects with asthma, IL-17 production increases in peripheral blood after allergen challenge. In addition to its proinflammatory role in asthma, in contrast to Th1, Th2, and Th17 cells, Treg cells act directly or indirectly to suppress the function of effector T cell responses. Treg cells have cytokine profiles that are distinct from Th1 and Th2 cells and appear to control development of autoimmune disease and are able to inhibit development of allergic Th2 responses. These cells originate in the thymus, require expression of the transcription factor FoxP3 for the development of the suppressive function, and do not produce cytokines (91, 92).
FIGURE 2.6: Development of effector T-cell subsets. Following antigen processing, linear T-cell epitopes are presented to CD4+ T cells by major histocompatibility complex (MHC) class II molecules. Naive T cells differentiate into Th1, Th2 and Th17 effector subsets. Their differentiation requires cytokines that are released from dendritic cells. T-cell activation in the presence of interleukin-4 (IL-4) enhances differentiation and clonal expansion of TH2 cells, perpetuating the allergic response. IL-12, IL-18 and IL-27 induce TH1 cell differentiation and IL-6 and transforming growth factor β (TGFβ) induce the differentiation of TH17 cells. After differentiation, T cells show clonal expansion induced by IL-2, IL-4, IL-15 and IL-21, which share a common cytokine receptor γ chain, in an autocrine and paracrine fashion. TCR, T-cell receptor (93).
FIGURE 2.6: Functions of Th1, Th2, Th17 and Treg cells. Although T regulatory (TReg) cells can efficiently inhibit the functions of three effector T-cell subsets, it seems that all four T-cell subsets counter-regulate each other and directly or indirectly promote the type of inflammation that they are committed to. The functions of T-cell subsets and the cytokines that mediate these functions are shown in the boxes. ECM, extracellular matrix; GM-CSF, granulocyte monocyte colony stimulating factor; IFNγ, interferon γ; IL, interleukin; TGFβ, transforming growth factor β (93).

2.8.8 Macrophages
Macrophages, which are derived from blood monocytes have the capacity to initiate a particular type of inflammatory response by the release of a certain types of cytokines. Depending on the stimulus macrophages may increase or decrease the inflammation.
Alveolar macrophages have a suppressive effect on lymphocyte function, but may be impaired in asthma after allergen exposure. In asthmatic patients there is reduction of an anti-inflammatory cytokines IL-10 from by alveolar macrophages. Macrophages in normal subjects inhibits the secretion of IL-5, probably via the release of IL-12, but this may impaired in patients with allergic asthma. Macrophages may therefore play an important anti-inflammatory role, by preventing the development of allergic inflammation. There may be subtypes of macrophages that perform different inflammatory, anti-inflammatory or phagocytic roles in allergic disease (94, 95).

2.8.9 Dendritic cells
Dendritic cells are specialised macrophage-like cells and has ability to induce a T-lymphocyte mediated immune response and therefore play a perilous role in the development of asthma. Dendritic cells in the respiratory tract acts as very effective antigen-presenting cells that play an important role in the initiation of allergen-induced responses in asthma (96). Dendritic cells take up allergens, process them to peptides and migrate to local lymph nodes where they present the allergenic peptides to uncommitted T-lymphocytes. Granulocytemacrophage colony-stimulating factor (GM-CSF), which is expressed in abundance by epithelial cells and macrophages in asthma, leads to differentiation and activation of dendritic cells. This leads to production of myeloid dendritic cells which favour the differentiation of T-helper (Th)2 cells (96).

2.8.10 Platelets
Several animal studies have documented the importance of platelets as participants in bronchoconstriction. It is well established that activated platelets secrete various bronchoconstricting mediators, such as histamine, serotonin (5-HT), platelet activating factor (PAF) and arachidonic acid metabolites. Intravenous administration of PAF, a phospholipid mediator released by a number of both inflammatory and non-inflammatory cells, to guinea pigs results in acute bronchoconstriction and recruitment of macrophages, eosinophils and platelets into the airways. As a potent activator of platelets and neutrophils, PAF, also known as acetyl glyceryl ether phosphorylcholine (AGEPC), can induce systemic anaphylaxis when given intravenously (97).
2.8.11 Adhesion Molecules

Adhesion molecules help adhesion of the various cells to each other and the tissue matrix to facilitate infiltration and migration of these cells to the site of inflammation. Adhesion molecules are found on a variety of cells, such as neutrophils, monocytes, lymphocytes, basophils, eosinophils, granulocytes, platelets, endothelial cells and epithelial cells, and can be activated by the many inflammatory mediators present in asthma. Adhesion molecules have other functions such as, promoting cell activation, cell-cell communication and cell migration and infiltration (98). A major role of adhesion molecules is in the recruitment of leukocytes from the vascular lumen to tissues. More than 35 adhesion molecules have been identified. The adhesion molecules are divided into various families on the basis of their chemical structure. Some of which thought to be important in inflammation includes; integrins, immunoglobulin supergene family, selectins and carbohydrate ligands including intracellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1. Many studies are indicate that adhesion molecules are upregulated in allergic inflammation and play a critical role in the pathogenesis of allergic inflammation (98).

2.8.12 Inflammatory Mediators

Many different types of inflammatory mediators involves in respiratory diseases which account for some of the pathologic features of inflammatory lung diseases. The various inflammatory mediators such as histamine, prostaglandins, and leukotrienes contract airway smooth muscle, increase microvascular leakage, increase airway mucus secretion, and attract other inflammatory cells (Fig. 2.7). Each mediator has many effects and many mediators have similar effects, so it is theoretically unlikely that antagonizing a single mediator will have a major impact in complex diseases such as asthma. The chronic inflammation is likely to be mediated by cytokines released within the airway wall and have critical role in orchestrating and perpetuating the chronic inflammation of asthma (13).
FIGURE 2.7: Inflammatory mediators in asthma. Multiple inflammatory cells release a whole range of inflammatory mediators, which then act on inflammatory receptors on target cells of the airway to produce the typical inflammatory effects. AHR, airway hyperresponsiveness; PAF, platelet-activating factor; sm, smooth muscle.

TABLE 2.2: Different inflammatory mediators and their effects

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Source</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>T cells</td>
<td>Increases production of IgE, increases number of Th2 cells</td>
</tr>
<tr>
<td>IL-5</td>
<td>T cells</td>
<td>Increases number of eosinophils</td>
</tr>
<tr>
<td>IL-9</td>
<td>T cells</td>
<td>Increases number of mast cells</td>
</tr>
<tr>
<td>IL-13</td>
<td>T cells, mast cells, basophils, eosinophils</td>
<td>Increases production of IgE, induces airway remodeling</td>
</tr>
<tr>
<td>IL-17</td>
<td>T cells</td>
<td>Increases neutrophil number, induces production of cytokines by airway epithelium</td>
</tr>
<tr>
<td><strong>Proinflammatory Cytokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Epithelial cells, macrophages, mast cells</td>
<td>Proinflammatory</td>
</tr>
<tr>
<td>IL-1</td>
<td>Epithelial cells, macrophages</td>
<td>Proinflammatory</td>
</tr>
<tr>
<td>TNF-</td>
<td>Epithelial cells, macrophages, mast cells</td>
<td>Proinflammatory</td>
</tr>
<tr>
<td>TSLP</td>
<td>Epithelial cells</td>
<td>Activates dendritic cells, increases number of Th2 cells</td>
</tr>
<tr>
<td><strong>Chemokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molecule</td>
<td>Source Cells</td>
<td>Recruitment</td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>CCL2</td>
<td>Epithelial cells, macrophages, T cells</td>
<td>Recruits monocytes, T cells, dendritic cells</td>
</tr>
<tr>
<td>CCL5</td>
<td>Epithelial cells, macrophages, T cells</td>
<td>Recruits T cells, eosinophils, basophils</td>
</tr>
<tr>
<td>CCL11</td>
<td>Epithelial cells, macrophages</td>
<td>Recruits eosinophils</td>
</tr>
<tr>
<td>CXCL8</td>
<td>Epithelial cells, macrophages, mast cells</td>
<td>Recruits neutrophils</td>
</tr>
</tbody>
</table>

**Growth Factors**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Source Cells</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>Epithelial cells, macrophages, T cells</td>
<td>Increases number of neutrophils and eosinophils</td>
</tr>
<tr>
<td>SCF</td>
<td>Epithelial cells, smooth muscle cells, fibroblasts, eosinophils</td>
<td>Increases number of mast cells</td>
</tr>
</tbody>
</table>

**Eicosanoids**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Source Cells</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukotrienes</td>
<td>Mast cells, eosinophils</td>
<td>Airway hyperactivity</td>
</tr>
<tr>
<td>PGD2</td>
<td>Mast cells</td>
<td>Airway hyperactivity</td>
</tr>
</tbody>
</table>

### 2.8.12.1 Histamine

Histamine plays a significant role in triggering an asthmatic reaction. Human lung is a rich source of histamine stored mainly in the secretory granules of mast cells which are widely distributed in human respiratory tract and located within the walls of airways and of alveoli. Histamine released during the early and late phase allergic reactions; (7) induces the secretion of mucus that cause extravasation of plasma proteins leading to airways mucosal oedema and contract the airway smooth muscle. Histamine may play a role in the immunomodulation of the IgE immune response as well as in the regulation of the airway inflammation by the activation of epithelial cells and macrophages and possibly by inducing smooth muscle hyperplasia (99).

### 2.8.12.2 Leukotrienes

The cysteinyl-leukotrienes LTC4, LTD4, and LTE4 are potent constrictors of human airways which has significant role in the acute asthma. These mediators contributes approximately half of the bronchoconstrictor responses to triggers, such as allergens and exercise. Moreover, LTD4 increases eosinophilic inflammation in the airways and LTB4 is a potent chemotactic agent for neutrophils and plays an important role in neutrophilic inflammatory diseases of the airways, such as COPD and cystic fibrosis (100).
2.8.12.3 Prostaglandins
Prostanoids (prostaglandins and thromboxane) are synthesized by the enzyme cyclooxygenase formed from arachidonic acid. Isoprostanes; a new class of prostanoids, are formed nonenzymatically via oxidation of arachidonic acid, and these mediators are useful markers of oxidative stress in lung diseases. The most prevalent of these, 8-isoprostane (8 epi-PGF2α), is a potent constrictor of human airways (101).

2.8.12.3 Platelet-Activating Factor
PAF has been attracted a considerable attention because it mimics many of the features of asthma, including smooth muscle hyperresponsiveness. Although PAF is produced by the inflammatory cells; a potent PAF antagonists such as apafant (WEB 2086) and modipafant in chronic asthma have been clinically disappointing. PAF may play a role in acute respiratory distress syndrome (ARDS) (102).

2.8.12.4 Endothelins
Endothelins are potent vasoconstrictors and bronchoconstrictors; peptide mediator which induced airway smooth muscle cell proliferation and fibrosis and may therefore play a role in the asthma (103).

2.8.12.5 Oxygen-Derived Free Radicals
Oxidative stress may play an important role in asthma and in COPD. Many inflammatory cells produce oxygen-derived free radicals such as superoxide anions. Hydrogen peroxide causes contraction of airway smooth muscle and changes in receptor function, which could influence airway reactivity and may activate NF-κB to increase the expression of multiple inflammatory genes. Oxidative stress may also impair the action of corticosteroids (104).

2.8.12.7 Adenosine
Adenosine is released from various cell types under conditions of stress, such as hypoxia. Adenosine has a potent bronchoconstrictor effect in asthmatic patients and releases histamine from “primed” mast cells via an α2b-receptor. Theophylline is an antagonist of adenosine receptors, but it is unlikely that this action accounts for its antiasthma effect, although it may underlie serious side effects such as cardiac arrhythmias and seizures (105).
2.8.12.8 Bradykinin
Bradykinin is a potent bronchoconstrictor in asthma and causes contraction of airways. It may be an important mediator of cough in asthma, because of capacity to sensitize afferent nerves to various activating stimuli (106).

2.8.13 Cytokines
Cytokines are small, extracellular signaling proteins usually less than 80 KD in size and many are glycosylated that play an important role in the co-ordination and persistence of inflammation in asthma. Cytokines are regulatory peptides and primarily derived from dendritic cells, mononuclear phagocytic cells, and antigen-presenting cells (APCs) are particularly effective in promoting the cellular infiltrate and damage to resident tissue characteristic of inflammation (Fig. 2.8). The major groups of cytokines includes Lymphokines, Proinflammatory cytokines, Inhibitory cytokines, Growth factors and Chemokine (107).

**FIGURE 2.8:** Actions of mononuclear phagocytic cell-derived cytokines. These cytokines are uniquely potent in generating the symptoms and initiating the immune responses associated with infection and inflammatory disorders. CNS, central nervous system; IL, interleukin; TNF, tumor necrosis factor; NK, natural killer; IFN, interferon; ICAM, intracellular adhesion molecule.
2.8.13.1 Th-2 Cell Derived Cytokines

Lymphokines are cytokines which are produced by T-lymphocytes which includes IL-2, IL-3, IL-4, IL-5, IL-7, IL-9, IL-15, IL-16 and IL-17 (107).

2.8.13.1.1 Interleukin-2

IL-2 is the short chain α-helical bundle with a size of 4-17 KD secreted primarily by Th0, Th1 cells and also synthesized by eosinophils and airway epithelial cells. It is a potent modulator of T-cell and NK cell function. The increased level of IL-2 found in bronchoalveolar lavage fluid of asthmatic patients (108).

2.8.13.1.2 Interleukin-3

IL-3 is a short chain α-helical bundle with molecular mass 20-26KD. IL-3 are produced by T-cells, NK cells and mast cells that stimulates the formation of mixed colonies of neutrophilic granulocytes, macrophages and erythrocytes. The mucosal biopsies and in bronchoalveolar lavage cells from patients with asthma has shown increased expression of IL-3 mRNA (109).

2.8.13.1.3 Interleukin-4

IL-4 is a short chain α-helical bundle with molecular weight 18 KD. The major cellular sources include thymocytes, mature T-cells, mast cells, basophils and CD4+ Th2 cells. IL-4 promotes the Th2 cell differentiation, IgE synthesis, mucus producing cells and fibroblast that involved in pathogenesis of airway remodeling (110).

2.8.13.1.4 Interleukin-5

IL-5 has molecular weight 45-50KD. Source of IL-5 includes activated T-helper cell populations, eosinophils, mast cells, CD4+ and CD8+ T-cells. Further, IL-5 induces activation and survival of eosinophils and eosinophil precursors (111).

2.8.13.1.5 Interleukin-7

IL-7 is produced by stromal cells of the thymus, bone marrow and keratin. IL-7 plays a key role in T-cell development. However there are no data specific to asthma (107).
2.8.13.1.6 Interleukin-9
IL-9 is 4-helix bundle cytokines with molecular mass of 14 KD. IL-9 is produced by T-cells, mast cells, eosinophils and neutrophils. Increased expression of IL-9 supports eosinophil infiltration and survival (112).

2.8.13.1.7 Interleukin-13
IL-13 is synthesized by activated T-lymphocytes, B-lymphocytes and mast cells. IL-13 has very similar biological activities to IL-4. There is a significant correlation between eosinophil counts and levels of IL-13. In allergen induced airway changes, IL-4 is crucial for the initial Th2 development during primary sensitization (113).

2.8.13.1.8 Interleukin-15
IL-15 promotes the activation, proliferation and cytokines release from various subsets of T-cells, NK-cells, mast cells and B-cells acts to be synergistic with IL-12 to induce proliferation of murine Th1 clones. Ryotaro et al. reported that over expression of IL-15 in vivo suppresses Th2-mediated-allergic airway response via induction of CD8+ T cell mediated Tc1 response (114).

2.8.13.1.9 Interleukin-16
IL-16 is produced by CD8+ T cells, epithelial cells, eosinophils and mast cells. Elevated levels of IL-16 have been found in BALF of asthmatic patients following allergen and histamine challenge (115).

2.8.13.1.10 Interleukin-17
IL-17 is a CD4+ T cell derived cytokine that stimulates NF-kB and IL-6 production in fibroblasts and co-stimulates T cell proliferation. It stimulates epithelial, endothelial, and fibroblastic cells to secrete cytokines such as IL-6, IL-8, GM-CSF, and PGE2 (116).

2.8.13.2 Proinflammatory Cytokines
Proinflammatory cytokines includes IL-1, TNF-α, IL-6, IL-11, GM-CSF and SCF which has important role in disease severity and resistance to anti-inflammatory therapy in asthma (117).
2.8.13.2.1 Interleukin-1
There are two distinct forms of IL-1 (α and β) with bared shaped structure and 17KD molecular weight. The IL-1 are produced by monocytes, macrophages, neutrophils, eosinophils, mast cells, platelets, lymphocytes, NK cells, endothelial cells, airway smooth muscle cells and vascular smooth muscle cells. Patients with symptomatic asthma showed increased level of IL-1 β in BALF compared with patients with asymptomatic asthma (107).

2.8.13.2.2 Tumor Necrosis Factor-α
TNF-α is produced by many cells including macrophages, T lymphocytes, mast cells, and epithelial cells, but the principal source is the macrophage. TNF-α being a proinflammatory cytokine is present abundantly in BALF of asthmatic patients (118).

2.8.13.2.3 Interleukin-6
IL-6 is secreted by monocytes, macrophages, T cells, B cells, fibroblasts, bone marrow stromal cells, keratinocytes, and endothelial cells. Human airway smooth muscle cells, upon activation with IL-1 β or TGF- β, released IL-6 (119).

2.8.13.2.4 Interleukin-11
IL-11, is produced by fibroblasts, epithelial cells and human airway smooth muscle cells when stimulated by IL-1 and TGF-β1. The elevated level of IL-11 found in BALF during upper respiratory viral infections (120).

2.8.13.2.5 Granulocyte Monocyte Colony Stimulating Factor (GMCSF)
GMCSF stimulates and regulate the growth, proliferation, maturation and function of hematopoietic cells. GMCSF is produced by macrophages, eosinophils, T-lymphocytes, fibroblast, endothelial cells, airway smooth muscle cell and epithelial cells. GM-CSF and IL-8 influences eosinophils chemoattraction play an important role in the etiology of bronchial asthma (121).

2.8.13.2.6 Stem cell factor (SCF)
SCF is produced by bone marrow stromal cells, fibroblasts and epithelial cells, such as nasal polyp epithelial cells. There is very little information on the expression of SCF in asthmatic airways (122).
2.8.13.3 Inhibitory Cytokines
Allergic airway inflammation not only induced by by expression of Th2 cytokines but also decreased expression of counter acting ones. Inhibitory cytokines includes IL-10, IL-12, IL-18 and IFN-γ.

2.8.13.3.1 Interleukin-10
IL-10 is long chain-α helical structure with molecular weight 34-40kD. IL-10 is produced by Th0, Th1, and Th2-like CD4+ T cell clones, cytotoxic T cells, activated monocytes and peripheral blood T cells including CD4+ and CD8+ T cells. IL-10 cytokine down regulate both Th1 and Th2-driven inflammatory processes and has beneficial effect on airway remodeling (123).

2.8.13.3.2 Interferon-γ (INF-γ)
The only known sources of IFN-γ are CD4+ and CD8+ T cells and natural killer cells. In asthmatic patients nebulized IFN-γ reduces the number of eosinophils in BAL fluid indicating its therapeutic potential in asthma. The reduced production of IFN-γ has been also reported in asthmatics. This suggests that defective IFN-γ production may be important in asthma although no polymorphisms of the IFN-γ gene have been associated with asthma (124).

2.8.13.4 Chemokines
Chemokines are small secreted proteins and chemotactic cytokines (8 to 10 kD) which attracts the leukocytes into tissues and regulate cell trafficking. They are classified into four subclasses: C, CC (β-chemokines), CXC (α-chemokines) and CX3C chemokines, based on the location of the first two cysteine residues in their sequence. The CC chemokines are involved in chemo attraction of eosinophils, monocytes, and T lymphocytes and are therefore has role in asthma. The CXC or α-chemokines principally attract neutrophils and have, therefore mainly been related to acute inflammatory processes. CC chemokines can be detected in bronchoalveolar lavage fluid, although only at low levels, even after the fluid has been concentrated (125).

2.8.13 Antibodies
An antibody, or an immunoglobulin (Ig), is a small protein molecule created by the immune system to have a close structural "fit" to the surface of a foreign substance. The foreign
substance is an antigen. The body manufactures five classes of antibodies, namely IgM, IgG, IgA, IgD, and IgE. Out of that IgE is found to be predominant in the development of the asthma (126).

2.8.13.1 Immunoglobulin E
IgE is the antibody responsible for activation of allergic reactions and is important to the pathogenesis of allergic diseases and the development and persistence of inflammation. IgE attaches to cell surfaces via a specific high-affinity receptor. The mast cell has large numbers of IgE receptors; these, when activated by interaction with antigen, release a wide variety of mediators to initiate acute bronchospasm and also to release pro-inflammatory cytokines to propagate underlying airway inflammation. Other cells, basophils, dendritic cells and lymphocytes also have high-affinity IgE receptors. Elevated level of IgE in both the serum and BALF is associated with bronchial asthma (126).

B) Phosphoinositide 3-kinases (PI3K)
Now a day research has focus on PI3K family which has an important role in inflammatory lung diseases particularly asthma which control inflammatory cells growth, differentiation, survival, migration, proliferation, and mediator production (19). Phosphatidylinositol (PtdIns) is phosphorylated on its myo-inositol ring to generate phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2); a precursor to other lipid signaling molecules. PtdIns (4,5)P2 is widely recognized as a potent messenger itself, and a major mediator of biochemical activities and cellular functions (19).

2.9 PI3K family
Phosphoinositide-3-kinases are a family of enzymes that play vital role in cellular signaling via their activation-dependent production of modified phosphatidylinositol. PI3K enzymes are subdivided into three classes according to their structure, lipid substrate specificity and regulation (127). Each type of PI3K contains a C2 domain and a catalytic domain connected by a helical domain (the ‘PIK domain’) that is found in lipid kinases but not protein kinases (128). This kinase domain interacts with Adenosine-triphosphate (ATP) and transfers a phosphate from ATP to Phosphatidylinositol 4,5-bisphosphate (PIP2) generating Phosphatidylinositol 4,5-trisphosphate (PIP3) (129). On the basis of activation mechanisms class I PI3K family divided into subclasses IA and IB. Class IA enzymes consist of three types of p110 catalytic subunit as p110α, p110β (expressed ubiquitously in many tissues and
organs) and p110δ (expressed primarily in leukocytes). Class IA enzymes also have five different regulatory subunits: p85α, p55α and p50α in that regulatory subunits, p85α is the most abundantly expressed (128, 130). The single class IB PI3K enzyme, p110γ, is present only in mammals and is expressed preferentially in leukocytes (127, 128). The class II PI3K family contains three members: PI3K-C2α and PI3K-C2β are expressed ubiquitously, whereas PI3K-C2γ is expressed primarily in hepatocytes (127, 128). In mammalian cells, class III PI3K is involved in the movement of proteins through the lysosome. How class III PI3K is activated in vivo is largely unknown (131).

TABLE 2.3: Characteristics of the PI3K family (19)

<table>
<thead>
<tr>
<th>PI3K Family</th>
<th>Isoforms</th>
<th>Catalytic Subunit</th>
<th>Regulatory Subunit</th>
<th>In vitro Substrate</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>PI3Kα</td>
<td>p110α</td>
<td>p85, p85β</td>
<td>PtdIns</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td></td>
<td>PI3Kβ</td>
<td>p110β</td>
<td>p55γ</td>
<td>PtdIns(4)P</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td></td>
<td>PI3Kδ</td>
<td>p110δ</td>
<td>p85α, p85β</td>
<td>PtdIns(4,5)P2</td>
<td>Whole blood, thymus</td>
</tr>
<tr>
<td>IB</td>
<td>PI3Kγ</td>
<td>p110γ</td>
<td>p101, p84/87</td>
<td>PtdIns(4,5)P2</td>
<td>Whole blood, thymus</td>
</tr>
<tr>
<td>II</td>
<td>C2α</td>
<td>Clathrin</td>
<td>PtdIns,PtdIns(4)P</td>
<td>Widely expressed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C3β</td>
<td>Clathrin</td>
<td>PtdIns</td>
<td>Widely expressed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C4γ</td>
<td>Clathrin</td>
<td>PtdIns</td>
<td>Prostate, Liver, Breast</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Vps34p</td>
<td>Vps15p (p150)</td>
<td>PtdIns</td>
<td>Ubiquitous</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Beclin 1</td>
<td>Constitutive</td>
<td></td>
</tr>
</tbody>
</table>

2.9.1 PI3K Signal Transduction

The Class IA enzymes are activated by tyrosine kinases (e.g. growth factor receptors), antigen receptors, and cytokines such as IL-2, IL-3, IL-6, IL-7, IL-15, granulocyte colony-stimulating factor, erythropoietin, oncostatin M and interferons (IFNs) including T cells and dendritic cells (DCs). Class IB PI3K is activated via chemokine receptors. Class II PI3Ks are thought to be activated by some tyrosine kinase receptors and chemokines while class III PI3K appears to be constitutively activated [Fig. 2.9] (128, 130, 132).
FIGURE 2.9: Signal transduction pathways involving PI3Ks in immune cells. Proteins containing PH domains (PKB, PDK1, Vav and PLC-γ) are present downstream of PI(3,4)P2 and PI(3,4,5)P3. Various target proteins function downstream of these molecules. For example, p70S6K is involved in cellular proliferation. Proteins containing FYVE and PX domains such as p40phox, function upon binding to PI(3)P and/or PI(3,4)P2. Activation mechanisms of class II and class III PI3Ks are largely unknown.

2.9.10 Downstream of PI3K signaling
Termination of PI3K signaling by degradation of PtdIns(3,4,5)P3 can be mediated by inositol 5-phosphatase (SHIP) and phosphatase and tensin homolog deleted on chromosome 10 protein (PTEN) [Fig. 2.10] (133). PTEN removes the 3-phosphate of PtdIns(3,4,5)P3 and, thus, directly counteracts all types of PI3K by catalyzing the opposite reaction. It was reported that PTEN over expression reduced airway hyperresponsiveness and vascular endothelial growth factor expression in a murine model of asthma. In contrast, SHIP removes the 5-phosphate from the inositol ring of PtdIns(3,4,5)P3 to generate PtdIns(3,4)P2, and this dephosphorylation of PtdIns(3,4,5)P3 by SHIP impairs downstream effects of PI3K (134).
FIGURE 2.10: PI3K signaling pathway. Activation of PI3K through tyrosine kinase or G-protein coupled receptors (GPCR). PtdIns(4,5)P2 converts into PtdIns(3,4,5)P3. Akt, PDK1, guanidine nucleotide factor (GEF), Rac/Rho kinase, Tec kinases and Btk are activated downstream of PI3K to carry out different cell functions e.g. cell growth, metabolism, angiogenesis, glucose uptake, survival, metabolism. PTEN dephosphorylates PtdIns(3,4,5)P3 and returns it to PtdIns(4,5)P2. SHIP can also dephosphorylate PtdIns(3,4,5)P3 to generate PtdIns(3,4)P2.

2.9.11 Role of PI3K in T cell differentiation
2.9.11.1 Th1 and Th2 cells
PI3K influences Th1 and Th2 differentiation through complex mechanisms. The signaling protein TOR (often referred to as mTOR, for mammalian (or mechanistic) target of rapamycin) is responsible for Th differentiation (135). TOR is encoded by a single gene in mammals (MTOR) but is the catalytic subunit of two distinct multi-protein assemblies known as TOR complex-1 (TORC1) and TOR complex-2 (TORC2). Both TORC1 and TORC2 contribute to Th differentiation. In mouse T cells, conditional knockout of mTOR completely blocks differentiation into Th1, Th2 or Th17 subsets (136).
2.9.11.2 Th17 cells
Th17 differentiation has been explored recently. PI3K inhibition blocks Th17 differentiation. A positive role for PI3K/AKT signaling in Th17 is supported by studies of T cells lacking TORC1 function. Mice with T cell-specific deletion of mTOR have a drastic reduction in Th17 differentiation (136) while rapamycin treatment suppressed Th17 differentiation (137).

2.9.11.3 Regulatory T cells
Forkhead box P3 (FOXP3)-expressing Treg cells have a pivotal role in the regulation of immune responses and in the maintenance of immunological self-tolerance. The mTOR has a central role in the regulation of the immune response and the PI3K–AKT–mTOR signaling pathway suppresses the induction of Foxp3 expression in vitro and in vivo (138). The upregulation of Foxp3 expression requires PTEN which antagonize PI3K function (139). Various studies demonstrated that application of the mTOR inhibitor rapamycin and its analogue everolimus resulted in a specific promotion of Foxp3+ Treg cell induction in vitro and in vivo. These drugs also increased the stability of Foxp3 expression and enhanced the survival of Treg cells (140).

2.9.12 Role of PI3K in various inflammatory cells
2.9.12.1 Neutrophils
The p110δ and p110γ isoforms of PI3K are markedly enriched in neutrophils. Class 1 PI3K control neutrophil spreading on endothelium, migration and pathogen killing (141). PI3K genetic knockout in mice results in fully differentiated neutrophils that fail to phosphorylate Akt and show impaired respiratory burst, motility and migration toward chemotactic stimuli, especially in vivo (142). In vitro study, p110δ specific inhibitor IC87114 caused the impairment of neutrophil movement. Moreover, neutrophils kill pathogens by generating reactive oxygen species (ROS) which impaired by genetic inactivation of p110γ and by p110β inhibition (142).

2.9.12.2 Mast cells
The release of mast cell is p110δ isoform of PI3K. Mice with inactive p110 and those treated with p110δ inhibitor are protected from passive cutaneous anaphylaxis. p110γ inhibition and p110γ−/− mice have normal early mast cell responses but a reduced phenotype at later stages, suggesting an impairment in signal amplification (143).
2.9.12.3 Dendritic cells

The p110γ−/− mice demonstrated normal differentiation but had markedly, impaired migration and reduction in severity of contact hypersensitivity and may delay symptoms in experimental autoimmune encephalitis. PI3K also promotes IL-6 release on stimulation of dendritic (144).

2.9.12.4 Macrophages

Macrophages initiates signaling through the p110δ and p110γ isoforms of PI3K. In a mouse genetic deletion of p110γ blocked C5aR signaling crucial for activation of lung macrophages. However C5a production occurred normally in p110γ−/− mice but was impaired in p110δ−/− mice, which demonstrated resistance to acute immune complex-induced lung injury, thus defining p110δ as a crucial element of FcγR signaling in the production of C5a (145).

2.9.12.5 Eosinophils

PI3K inhibitor wortmannin impaired eosinophil chemotaxis, its release from the bone marrow and reduction in eosinophilia in allergen-induced bronchial inflammation (146). Subsequent studies suggest that p110γ is the isoform most likely mediating this effect (22).

**FIGURE 2.12:** Schematic representation of the roles of PI3Kδ and PI3Kγ signaling in selected cells important in respiratory disease. ROS, reactive oxygen species; GC, glucocorticoid (147).
2.9.13 Role of PI3K in Asthma

PI3K signaling play significant role in mast cell and eosinophil function (20). Inhibition of PI3K signaling with LY294002 a nonselective inhibitor in an ovalbumin (OVA)-challenged murine model of asthma reduced inflammatory cell influx into the lung, reduction of IL-5, IL-13 and CCL11 (eotaxin) and suppressed tissue eosinophilia, airway mucus production and airway hyper-responsiveness [AHR] to inhaled metacholine (21, 148). In similar line a selective PI3Kδ inhibitor IC87114 in models of allergic inflammation attenuated vascular leakage, tissue eosinophilia, airway mucus production, release of cytokines (IL-4, -5 and -13), chemokines (CCL5, CCL11) and adhesion molecules (ICAM-1, VCAM-1) and IgE levels (134, 149).

PI3Kγ signaling may be important in mast cell degranulation and the amplification of the mast cell response (150). In addition, mast cells derived from the bone marrow of PI3Kγ knockout mice display reduced degranulation and calcium responses upon IgE receptor cross-linking and are protected from anaphylaxis following injection of IgE and allergen (151). Furthermore, PI3Kγ knockout mice have impaired eosinophil recruitment and survival in an OVA challenge model of allergic inflammation (22). Taken together, these studies clearly show that selective pharmacological inhibition of PI3Kδ/γ isoforms is a potential therapeutic strategy for suppressing allergic inflammation in asthma.

2.9.14 PI3K Inhibitors

Wortmannin is an irreversible inhibitor of PI3-Kinases by alkylating a lysine residue at the putative ATP binding site of p110 (23). Wortmannin binds the p110 kinase domain ATP-binding site, positioning itself in a conserved pocket using conserved p110α residues Ile 800, Ile 848, Val 850, Val 851, Ser 919, Met 922, Phe 930, Ile 932 and Asp 933. Wortmannin forms a covalent bond with Lys 802 and hydrogen bonds with Asp 933, Tyr 836, Val 851 and Gln 859 Inhibition of the ATP binding site prevents binding of ATP and subsequent transfer of the γ-phosphate group of ATP to PIP2. (152) LY294002 is a competitive inhibitor of ATP. Due to their instability and lack of selectivity leading to toxicity, neither wortmannin nor LY294002 are not valid pharmaceutical therapeutics (153). That being said, these compounds along with Quercetin, Myricetin and Staurosporine, can serve as excellent tools for investigating PI3K structure. Further, derivatives of wortmannin with more favorable pharmacological profiles are currently in clinical trials for various PI3K associated diseases (154).
2.10 Management of asthma

The aims of asthma management are to:

- Avoid causative and trigger factors
- Abolish symptoms and achieve a normal lifestyle
- Restore normal (or best possible) lung function
- Reduce the risk of severe attacks
- Optimize treatment and minimize side-effects of drugs

2.10.1 Medications of Asthma

The goal of asthma treatment is to achieve and maintain clinical control. Medications to treat asthma can be classified as controllers or relievers. **Controllers** are medications taken daily on a long-term basis to keep asthma under clinical control chiefly through their anti-inflammatory effects. They include inhaled and systemic corticosteroids, leukotriene modifiers, and long acting inhaled β2-agonists in combination with inhaled corticosteroids, sustained-release theophylline, cromones, and anti-IgE. Inhaled corticosteroids are the most effective controller medications currently available. **Relievers** are medications used on an as-needed basis that act quickly to reverse bronchoconstriction and relieve its symptoms. They include rapid-acting inhaled β2-agonists, inhaled anticholinergics, short-acting theophylline, and short-acting oral β2-agonists (24).

**Controller Medications**

2.10.1 Inhaled and Systemic corticosteroids

Corticosteroids are the most effective therapy available for patients with asthma which may be administered either orally or by inhalation (155). Various reports have shown that inhaled corticosteroids improves asthma symptoms more effectively than any other single long-term control medication in both children and adults (81). Mild to moderate persistent asthmatic patients when treated with inhaled corticosteroids has shown improved symptom like scores, lower exacerbation rates, and reduced symptom frequency (25).

Long-term oral corticosteroids therapy (that is, for periods longer than two weeks) may be required for severely uncontrolled asthma, but its use is limited by the risk of significant adverse effects. The therapeutic index (effect/side effect) of long-term inhaled corticosteroids is always more favorable than long-term systemic corticosteroids in asthma.
If oral corticosteroids have to be administered on a long term basis, attention must be paid to measures that minimum the systemic side effects (24).

### 2.10.1.1 Molecular Mechanisms

Inhaled corticosteroids are highly lipophilic, after entering in the airway cells; binds to cytosolic receptors. The glucocorticoids receptor complexes then move quickly into the nucleus where it binds to the glucocorticoid-responsive elements of genes, thereby either increasing or decreasing gene transcription. The altered transcription of many different genes is involved in the anti-asthma effect of glucocorticoids and inhibits transcription of the genes for the cytokines implicated in asthmatic inflammation. Glucocorticoids increase the synthesis of lipocortin-1 that has an inhibitory effect on phospholipase A (PLA), and therefore may inhibit the production of lipid mediators (156). Effects of glucocorticoids on gene transcription are shown in table.

**TABLE 2.4:** Effect of Corticosteroids on Gene Transcription

<table>
<thead>
<tr>
<th>Increased transcription</th>
<th>Annexin-1 (lipocortin-1, phospholipase A2 inhibitor)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β 2-adrenergic receptor</td>
</tr>
<tr>
<td></td>
<td>Secretory leukocyte inhibitory protein</td>
</tr>
<tr>
<td></td>
<td>Clara cell protein (CC10, phospholipase A2 inhibitor)</td>
</tr>
<tr>
<td></td>
<td>IL-1 receptor antagonist</td>
</tr>
<tr>
<td></td>
<td>IL-1R2 (decoy receptor)</td>
</tr>
<tr>
<td></td>
<td>IkBα (inhibitor of NF-κB)</td>
</tr>
<tr>
<td></td>
<td>IL-10 (indirectly)</td>
</tr>
<tr>
<td>Decreased transcription</td>
<td>Cytokines: IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-11, IL-12, IL-13, IL-16, IL-17, IL-18, TNF-α, GM-CSF, SCF</td>
</tr>
<tr>
<td></td>
<td>Chemokines: IL-8, RANTES, MIP-1α, MCP-1, MCP-3, MCP-4, eotaxin</td>
</tr>
<tr>
<td></td>
<td>Adhesion molecules: ICAM-1, VCAM-1, E-selectin</td>
</tr>
<tr>
<td></td>
<td>Inflammatory enzymes: Inducible nitric oxide synthase, Inducible cyclooxygenase, Cytoplasmic phospholipase A2</td>
</tr>
<tr>
<td></td>
<td>Inflammatory receptors: Tachykinin NK1-receptors, NK2-receptors, Bradykinin B2-receptors</td>
</tr>
<tr>
<td></td>
<td>Peptides: Endothelin-1</td>
</tr>
</tbody>
</table>
FIGURE 2.13: Classical model of glucocorticoid action. The glucocorticoid enters the cell and binds to a cytoplasmic glucocorticoid receptor (GR) that is complexed with two molecules of a 90 kDa heat shock protein (hsp90). GR translocates to the nucleus where, as a dimer, it binds to a glucocorticoid recognition sequence (GRE) on the 5′-upstream promoter sequence of glucocorticoid-responsive genes. GREs may increase transcription and negative (n) GREs may decrease transcription, resulting in increased or decreased mRNA and protein synthesis (156).

Corticosteroids have direct inhibitory effects on many inflammatory cells that involved in asthma such as macrophages, T-lymphocytes, eosinophils, and airway epithelial cells and reduces the number of mast cells within the airway (26, 28). Corticosteroids may also inhibit plasma exudation and mucus secretion in inflamed airways (157). By reducing airway inflammation, inhaled glucocorticoids consistently lessen airway hyperresponsiveness in adults and children with asthma (158). Inhaled glucocorticoid therapy not only makes the airways less sensitive to spasmogens, but also limits the maximal narrowing of the airway in response to a spasmogen (159). The reduction in airway hyperresponsiveness may not be maximal until treatment has been given for several months. The magnitude of the reduction varies, and airway responsiveness often remains abnormal. Although the treatment
suppresses inflammation, it may be unable to reverse the persistent structural changes that underlie the disease.

![Image](image.png)

FIGURE 2.14: Cellular effect of corticosteroids (160).

### 2.10.2 Adverse effects of corticosteroids
#### 2.10.2.1 Glucose Intolerance
Corticosteroids are the most common cause of drug-induced diabetes. Though the exact prevalence is not known, a few observations suggest that corticosteroids-induced diabetes or hyperglycemia is common:

- 52.9% were developed diabetes in the first 3 months and 14.5% after 1 year after initiation of systemic glucocorticoids (prednisolone-equivalent glucocorticoid dose exceeding 20 mg/day for at least 4 weeks) (161)
- nearly 9% of patients with rheumatoid arthritis have been shown to develop diabetes 2 years after starting glucocorticoid treatment (162);
- 42% of nondiabetic patients with primary renal disease treated with prednisolone 0.75mg/kg/day were found to have 2-hour post-lunch plasma glucose concentrations higher than 200mg/dL but normal fasting glucose levels. The authors also reported that more than 50% of patients developed diabetes or impaired glucose tolerance 10
weeks after starting the treatment with prednisone (mean dose 10mg/day); however, one of the limits of this study was the small number of patients (163).

- In a case-control study, the odds ratio (OR) of starting an oral hypoglycaemic agent or insulin in patients receiving a hydrocortisone-equivalent dose of 1 to 39mg/day was 1.77; patients receiving higher doses of glucocorticoids have been shown to have higher ORs: 3.02 for 40 to 79mg/day, 5.82 for 80 to 119mg/day, and 10.34 for 120mg/day or more (164).

The long term use of corticosteroids causes hyperglycemia which may related to the inhibition of glucose metabolism or insulin resistance mainly interfere insulin signals cascade and inhibitory effect on β cells (32). Glucocorticoids increase gluconeogenesis and blood glucose increases by 10–20% (3). Risk factors for the development of corticosteroid-induced hyperglycemia include steroid dose and patient age, body weight, and family history of diabetes (165).

### 2.10.2.2 Hyperlipidemia

Besides insulin resistance, visceral obesity is another major side effect of corticosteroids treatment. Corticosteroids have been found to be both lipolytic as well as adipogenic (34) and regulate differentiation, function and distribution of adipocytes (166). High dose of corticosteroids treatment has been associated with increased waist circumference, triglycerides, total and LDL-cholesterol levels all symptoms increasing the risk for cardiovascular diseases. However, the effects of corticosteroids on HDL-cholesterol are contradictory; in a study population of 15000 Americans, corticosteroids were associated with increased HDL-cholesterol ratio in the >60 years population, implying an improved lipid profile (167). The effects of corticosteroids on lipid metabolism might be due to VLDL secretion and lipoprotein lipase (LPL) activity (168). Corticosteroids also increases the activity of acetyl-coenzyme A carboxylase and free fatty acid synthetase, down regulation of LDL receptor activity, increase in the activity of HMG-CoA reductase and inhibition of lipoprotein lipase (35).

### 2.10.2.3 Psychiatric adverse effects

Long term use of corticosteroids are associated with alteration of mood, insomnia, hyperactivity and even frank psychosis (37, 169). The prevalence has been variably described as occurring in between 2% to 57% of corticosteroid-treated patients (38). There
are several case reports that suggest psychiatric disturbances may occasionally occur with steroid inhalers. A 5-year-old child with asthma developed symptoms of mania including agitation, irritability, and insomnia 2 days following the addition of inhaled budesonide at 200 g/day. The psychiatric symptoms observed in the child resolved with dose reduction (170). Phelan (171) found that a 69-year-old man who had previously developed protracted manic symptoms with oral prednisolone became euphoric with pressured speech and visual hallucinations after receiving 400 g/day of inhaled beclomethasone for 3 weeks. Similarly, a bipolar disorder patient who was stable on lithium therapy promptly developed severe mania requiring hospitalization after the addition of a beclomethasone inhaler (eg, 1 puff prn) for asthmatic symptoms (172). Inhaled corticosteroids are widely used, thus, the paucity of case reports of psychiatric symptoms associated with their use suggests that severe reactions are uncommon. The available data show that patients undergoing chronic, long-term corticosteroid therapy may have an increase in depressive symptoms while acute steroid therapy may be primarily associated with mania (172). Psychotic reactions, develop most frequently within the first 5 days of use of corticosteroids (39) patients that receive less than 40 mg of prednisone daily or the equivalent have minimal risk for development of severe psychiatric illnesses (173). It has been postulated that steroid psychosis may result from the induction of tyrosine hydroxylase synthesis (39) or via direct disruption of hippocampus-dependent behavior by some as yet unknown mechanism (38). Moreover, corticosteroid use has also been associated with both cerebral and hippocampal atrophy, which may have a pathologic role. The incidence is unknown but is not infrequently associated with the use of large doses (60–100 mg/d of prednisone or equivalent) and usually improves after dose tapering or steroid discontinuation (174).

2.10.2.4 Osteoporosis

Osteopenia results from corticosteroid use is a significant risk for the occurrence of vertebral fractures (175). Corticosteroids inhibits the bone formation directly by inhibiting osteoblast differentiation and type I collagen synthesis and indirectly by inhibition of calcium absorption and enhancement of urinary calcium excretion (176). Calcium absorption is decreased due to decreased in renal hydroxylation/activation of the vitamin D and a vitamin D–independent defect in transmucosal calcium transport and increased in calcium excretion because of a corticosteroid-mediated defect in renal tubular calcium reabsorption, as well as volume expansion (177, 178). The incidence of fracture in steroid treated individuals is
between 10% and 20% and those at particular risk are: under 15 years and over 50 years, post-menopausal or amenorrhoic women, slim build and limited mobility (179).

2.10.2.5 Hematopoietic/Immunologic Side Effects
Corticosteroids increases the total peripheral leukocyte count because of an increase in neutrophils as they are shifted from the marginating pool to the circulating pool. The leukocyte count, which may approach 14,000 to 20,000/mm3, generally returns to normal within 1 week of steroid discontinuation (180).

2.10.2.6 Gastrointestinal Disease
A meta-analysis of 6,602 patients in 93 studies showed that corticosteroid use was very rarely associated with peptic ulcer formation (181). Some large studies have found weak associations between corticosteroid use and peptic ulcer disease, whereas others have found none at all (182). However, preexistent peptic ulcer disease may be worsened by use of corticosteroid which might be due to coexistent nonsteroidal anti-inflammatory drug use, cumulative corticosteroid dose and duration of use, and previous history of peptic ulcers (183).

2.10.2.7 Myopathy
During corticosteroid use there is a reduction in muscle protein synthesis and protein catabolism; therefore, muscle weakness and loss of bulk can occur. In its extreme form a steroid myopathy may develop, affecting the proximal muscles. This can be severe enough to affect mobility and is easily demonstrated by asking patients to stand from sitting without using their hands. Should a myopathy develop the steroid dose should be minimized and the use of steroid sparing agents considered. Muscle weakness can also occur as a result of hypokalaemia; electrolytes should therefore be checked in this situation (179).

2.10.2.8 Ophthalmologic
The risk of glaucoma increased by corticosteroids by increasing the intraocular pressure. Patients with age of >40 years and those with diabetes or myopia, family history of glaucoma appear to be at increased risk (184, 185). It has been postulated that hyaluronic acid gets accumulated in the presence of corticosteroids and prevents aqueous humor outflow. Typically, intraocular pressure returns to normal within 2 to 4 weeks after the
discontinuation of corticosteroids, although irreversible intraocular pressure has been reported (186).

**TABLE 2.5:** Tissue-Specific Side Effects of High-Dose or Prolonged Glucocorticoid Therapy (187)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal gland</td>
<td>Adrenal atrophy, Cushing’s syndrome</td>
</tr>
<tr>
<td>Cardiovascular system</td>
<td>Dyslipidemia, hypertension, thrombosis, vasculitis</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>Changes in behavior, cognition, memory, and mood (i.e., glucocorticoid-induced psychoses), cerebral atrophy</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>Gastrointestinal bleeding, pancreatitis, peptic ulcer</td>
</tr>
<tr>
<td>Immune system</td>
<td>Broad immunosuppression, activation of latent</td>
</tr>
<tr>
<td>Integument</td>
<td>Atrophy, delayed wound healing, erythema, hypertrichosis, viruses, perioral dermatitis, petechiae, glucocorticoid-induced acne, striae rubrae, distensae, telangiectasia</td>
</tr>
<tr>
<td>Musculoskeletal system</td>
<td>Bone necrosis, muscle atrophy, osteoporosis, retardation of longitudinal bone growth</td>
</tr>
<tr>
<td>Eyes</td>
<td>Cataracts, glaucoma</td>
</tr>
<tr>
<td>Kidney</td>
<td>Increased sodium retention and potassium excretion</td>
</tr>
<tr>
<td>Reproductive system</td>
<td>Delayed puberty, fetal growth retardation, hypogonadism</td>
</tr>
</tbody>
</table>

2.10.2 Leukotriene Receptor Antagonists
Montelukast and zafirlukast are most widely available leukotriene receptor antagonists and became alternative therapies to mild persistent asthma in patients who are unable or unwilling to use inhaled corticosteroids (81). Leukotriene receptor antagonists has several advantages like ease of use, high rates of compliance and they can provide good control of asthma symptoms in many patients (81).

2.10.3 Long-Acting Beta$_2$ Agonists
Salmeterol and formoterol are available as long acting beta-2 agonist with duration of action >12 hours. Due to specificity of $\beta_2$-adrenergic receptors; they have low rates of tremor, palpitations or tachycardia (188) but regular use results in only mild tachyphylaxis (189).
2.10.4 Cromolyn Sodium and Nedocromil
Cromolyn sodium and nedocromil are mast cell stabilizers that interfere with chloride channel function. They are not preferred medication for the treatment of asthma as more safer of newer agents available (190).

2.10.5 Immnomodulators
Omalizumab is a monoclonal antibody that prevents binding of IgE to the high-affinity receptors on basophils and mast cells. It reduces the need for both oral and inhaled steroids (191).

2.10.6 Methylxanthines
Sustained-release theophylline used as an alternative but not preferred therapy with inhaled corticosteroids. Theophylline has mild anti-inflammatory activity and monitoring of serum theophylline level is essential (81).

2.10.7 Fast-Acting Agents
2.10.7.1 Inhaled Short-Acting Beta-2 Agonists
Short-acting beta-2 agonists are the most effective therapy for prompt relief of asthmatic symptoms. Albuterol, levalbuterol and pirbuterol are the most commonly used short-acting beta-2 agonists have onset of action of five minutes or less, peaking within 30 to 60 minutes, and a duration of action of four to six hours. Regular use (i.e., four or more times daily) does not affect potency but is associated with a reduction in the duration of action (192).

2.10.7.2 Oral Short-Acting Beta\textsubscript{2} Agonists
Oral short-acting beta\textsubscript{2} agonists are less potent, take longer to act, and have more side effects compared with inhaled short acting beta\textsubscript{2} agonists (192). Their use is strongly discouraged. Anticholinergic bronchodilators, such as ipratropium are not recommended as monotherapy for quick relief of asthmatic symptoms. They have a longer onset of action (20 to 30 minutes) and cause less bronchodilation than inhaled beta\textsubscript{2} agonists. Anticholinergic agents combined with short-acting beta\textsubscript{2} agonists, however, may be beneficial in treating severe asthmatic attacks or those induced by beta blockers (192).
2.10.8 The 5-lipoxygenase-activating protein (FLAP) inhibitor
Leukotrienes have physiological roles in innate immune responses and pathological roles in inflammatory diseases, such as asthma, allergic rhinitis and atherosclerosis. Anti-leukotriene therapy has proven benefits in the treatment of respiratory disease, either through the inhibition of leukotriene synthesis or the selective antagonism of leukotriene receptors. The first committed step in the synthesis of leukotrienes is the oxidation of arachidonic acid (AA) by 5-lipoxygenase (5-LO), and the integral membrane protein 5-lipoxygenase-activating protein (FLAP) is an essential partner of 5-LO for this process. FLAP is necessary in synthesis of leukotriene, which are lipid mediators of inflammation that is involved in respiratory and cardiovascular diseases (193).

2.10.9 Chemokine receptor inhibitors
More than 50 different chemokines are now recognized to be involved in the recruitment of inflammatory cells via the activation of more than 20 different surface receptors. Chemokine receptors are G-protein coupled receptors, which makes them amenable to small-molecule inhibitors an approach that has not yet proved feasible for classical cytokine receptors. Another strategy is to use antibodies, which can produce a long duration of blockade and avoid some of the toxicity issues associated with many small-molecule inhibitors. Some chemokine inhibitors seem to be selective for single chemokines, whereas others are promiscuous and mediate the effects of several related chemokines. Inhibitors of CCR4 and CCR8 might inhibit the recruitment of Th2 cells and persistent eosinophilic inflammation in the airways (2).

2.10.10 Kinase inhibitors
There has been particular interest in the p38 mitogen activated protein (MAP) kinase pathway, which is involved in expression of several inflammatory proteins that are relevant to asthma. p38 MAP kinase is blocked by a novel class of drugs, the cytokine suppressant anti-inflammatory drugs (CSAIDs), which include SB203580, SB239063 and RWJ67657. These drugs inhibit the synthesis of many inflammatory cytokines, chemokines and inflammatory enzymes. Interestingly, they seem to have a preferential inhibitory effect on synthesis of Th2 compared with Th1 cytokines, indicating their potential application in the treatment of atopic diseases. Furthermore, p38 MAP kinase inhibitors decrease eosinophil survival by activating apoptotic pathways125 and several inhibitors of p38 MAP kinase are
now in Phase II development. p38 MAP kinase is also involved in corticosteroid resistance in asthma (2).

2.10.11 Selective glucocorticoid receptor modulators (SGRM)
Glucocorticoids are widely used to suppress inflammation and treat various immune-mediated diseases. Some glucocorticoid receptor (GR)-regulated genes mediate the therapeutic response, whereas others cause debilitating side effects. Selective modulators that caused GR to regulate only a subset of its target promoters. Some compounds selectively inhibited GR-mediated gene activation without altering the repression of cytokine expression by GR. This approach will facilitate identification of genes and small molecules that augment beneficial effects of GR and diminish deleterious ones (194).

2.10.12 Phosphoinositide 3 kinase inhibitor
The PI3K/AKT/mTOR pathway is an important signaling pathway for many cellular functions such as growth control, metabolism and translation initiation. A Phosphoinositide 3-kinase inhibitor (PI3K inhibitor) is a potential medical drug that functions by inhibiting a Phosphoinositide 3-kinase enzyme which is part of this pathway and therefore, through inhibition, often results in tumour suppression (2).

2.10.12 Toll-like receptor 7 agonist
TLR7 is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity. The various TLRs exhibit different patterns of expression. This gene is predominantly expressed in lung, placenta, and spleen. Agonist activation of TLR7 stimulates an innate immune response leading to down-regulation of the Th2 adaptive response to allergen (195).

2.10.13 Urotensin antagonist
Urotensin-II (U-II) is an 11-amino acid cyclic peptide found in diverse species, including humans. U-II is most widely known for its ability to regulate smooth muscle tone. Compounds that antagonize the Urotensin-II receptor may be useful in the treatment of congestive heart failure, stroke, ischemic heart disease (angina, myocardial ischemia),
cardiac arrhythmia, hypertension (essential and pulmonary), COPD, restenosis and asthma (196).

2.11 Unmet Need (197)

A. Diagnosis

Some people with asthma symptoms may never receive a diagnosis and thus do not have the opportunity for good treatment and control.

B. Treatment

- Cost of medicines: people with asthma may not receive sufficient medication to control their asthma.
- Treatments prescribed may not be corresponding to those recommended guidelines.
- The underuse of inhaled glucocorticosteroids for long-term management
- **Resistance to Glucocorticoid:** Asthma/COPD can be controlled with low doses of inhaled corticosteroids, which have now become first-line treatment for most patients but the numbers of asthmatic patients are becoming resistant to glucocorticoid. Clinically, Glucocorticoid-resistant asthma is defined as failure to improve lung function; forced expiratory volume in 1 second (FEV₁) by >15% after treatment with 30 to 40 mg of prednisolone for 2 weeks. Steroid resistance asthma might be a significant problem, even the availability of highly potent new inhaled steroids.
  
There may be several mechanisms for resistance to the effects of glucocorticoids which includes a high level of IL-2 and IL-4; reduction in Tregs, decrease in steroid receptor number and/or binding capacity, increase in glucocorticoid receptor beta isoform (GR-β), defective histone acetylation, transcription factor activation such as AP-1 and increase in P-glycoprotein activity.
TABLE 2.6: Proposed mechanisms of corticosteroid resistance in asthma

- Genetic abnormalities in glucocorticoid receptors (GRs)
- Pharmacokinetic abnormalities (drug effects)
- Lipocortin-1 antibodies
- Effects of Th2 cytokines (interleukin [IL]-2 + IL-4, IL-13)
- Increased GR-β
- p38 MAP kinase activation
- Reduced IL-10 secretion
- Increased activation of AP-1 (activation of Jun-N terminal kinase)
- Abnormalities in histone acetylation
- Oxidative stress and cigarette smoking
- Latent viral infections

2.12 The need for new therapies

Combinations of inhaled corticosteroids and long-acting β2 agonists are effective in most (about 90%), but not all, asthmatic individuals (198). Indeed, even patients whose asthma is apparently well controlled by existing therapies might benefit from more efficacious therapies that are easier to comply with it (199). Improved compliance in the mild-to-moderate asthmatic patient could be achieved by the development of safe oral versions of conventional treatments or of new, more efficacious treatments, particularly if these agents altered the course of the disease or pointed towards a cure.

Recently, newer physiologic functions for vitamin D have been identified. Epidemiologic and genetic studies as well as research using animal models suggest vitamin D plays a vital and complex role in immune system function and regulation. Multiple immune cell types express vitamin D receptors, including activated T and B cells, macrophages and dendritic cells. Vitamin D inhibits the function of T lymphocytes both directly and via effects on antigen presenting cells (APCs). It has potent antiproliferative effects on CD4+ T cells. Moreover, numerous clinical studies have shown that low vitamin D level (vitamin D deficiency) associated with asthma exacerbation and supplementation with vitamin D has significant improvement in asthma symptoms (49).
C) Vitamin D
Vitamin D is a fat-soluble seco-sterols and is stored in the liver and fat tissue of humans. Vitamin D has two physiological forms (Fig 2.15): ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) which differ in their side chain structure but have equal biological activity (42). Vitamin D2 is made from the ultraviolet irradiation of ergosterol and also found in plants, yeast and fungi. Vitamin D3 occurs naturally in the skin of many animals including humans; produced through ultraviolet light exposure of 7-dehydrocholesterol. Vitamin D3 also manufactured through ultraviolet irradiation of 7-dehydrocholesterol from lanolin (42).

![Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol)](image)

**FIGURE 2.15:** Forms of Vitamin D

Vitamin D promotes the absorption of dietary calcium and phosphorus in the small intestine and mobilizes these mineral stores from the bone, playing a critical role in skeletal development and cellular functions (200). Observational epidemiological data has suggested an association between vitamin D in the body and the risk of health problems including asthma, cancer, cardiovascular disease, diabetes and metabolic syndrome, falls and physical performance, immune functioning and autoimmune disorders, infections, neuropsychological functioning, and preeclampsia in adults (200).

**2.13 Sources and Metabolism of Vitamin D**
Human being gets vitamin D from diet, dietary supplements or exposure to sunlight. The major source of vitamin D are sunlight which exposed to skin between the 10:00 to 15:00 hours in all season (42, 201). Vitamin D3 is produced in the skin from 7-dehydrocholesterol
by solar ultraviolet B radiation of wavelength 290-315 nm, which breaks the B ring to form pre-vitamin D3. Previtamin D3 rapidly isomerizes by heat dependent process to vitamin D3. An excess previtamin D3 or vitamin D3 is destroyed by sunlight to inactive photoproducts but excessive exposure does not cause vitamin D3 intoxication (42). Vitamin D made in skin or from dietary supplements are stored in both adipose and muscle (202) and release form fat cells (203). Vitamin D from the skin and dietary supplements are enters the blood circulation and bind to vitamin D binding protein (DBP) which carries vitamin D to the liver and kidney for biotransformation (204).

In the liver vitamin D metabolized to 25-hydroxyvitamin D3 (25(OH)D3) by cytochrome P450 vitamin D 25 hydroxylases enzyme (204). The 25(OH)D3 is the major circulating form of vitamin D with long half-life of 15 days (205) that is used by clinicians to determine patients vitamin D status (42). In proximal tubule of kidney, 25(OH)D3 metabolized to active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) which is responsible all of the biological actions of vitamin D. The renal production of 1,25(OH)2D3 can be regulated by level of calcium, phosphorus and parathyroid hormone (42, 204). The cytochrome P450 monooxygenase 25(OH)D 1α hydroxylase (CYP27B) metabolizes 25(OH)D3 to 1,25(OH)2D3 which present predominantly in kidney. This enzyme is also found in extrarenal sites including placenta, monocytes and macrophages (206).

The 1,25(OH)2D3 has very short half-life of <15 hours and catabolised to inactive form of vitamin D, 1,24,25(OH)3D3 with help of cytochrome P450, 24 hydroxylase. Further oxidation reaction of these catabolic metabolites result in water soluble calcitroic acid which excreted through kidney (204).
Synthesis of 1,25(OH)$_2$D$_3$

![Synthesis of 1,25(OH)$_2$D$_3$](image)

**FIGURE 2.16:** Synthesis and elimination of 1,25(OH)2D3

Elimination of 1,25(OH)$_2$D$_3$

![Elimination of 1,25(OH)$_2$D$_3$](image)

2.14 Factors Influencing Vitamin D Synthesis

A. Cutaneous

2.14.1 UVB radiation

Earth’s atmosphere, solar zenith angle, ozone (O$_3$), nitrogen, aerosols, water vapour, particulate pollutants and cloud matter; alter the amount of UVB radiation entering the skin and may significantly affect vitamin D$_3$ production.

2.14.2 Latitude

Below the latitude of approximately 35° North, UVB radiation is sufficient for vitamin D$_3$ synthesis all year round. However, at higher latitudes, vitamin D$_3$ is not produced during the winter months (207).
2.14.3 Cloths
Bleach and unbleached cotton cloth transmit approximately 24% and 14.4% of incident UV radiation whereas black wool reduced UVB irradiance by 98.6%, while white cotton reduced it by only 47.7%. (208)

2.14.4 Sunscreen
Topical sunscreen may absorb, reflect or scatter the incident UV radiation. A sunscreen with a sun protection factor (SPF)-8 and 15 reduces the skin's production of vitamin D by 95% and 99% respectively (209).

2.14.5 Melanin
Melanin acts like a sun-block and high content reduced epidermal vitamin D3 photosynthesis. Therefore, dark-skinned individuals may require extra vitamin D to avoid deficiency (210).

2.14.6 Age
Increase in age associated with decline of 7-dehydrocholesterol content in skin. The average concentration of 7-dehydrocholesterol in the epidermis of 77–88-year-olds is 65% lower then that in 21–29-year-olds (211)

2.14.7 Skin temperature
Isomerization of previtamin D3 to vitamin D3 is a temperature-dependent process. At 37°C, the T1/2 for the conversion of previtamin D to vitamin D in human skin decreases to 2.5 h (212).

B. Bioavailability
2.14.8 Gastrointestinal absorption of vitamin D
Any process resulting in malabsorption of intestinal fat may impair the absorption of vitamin D. The conditions in which vitamin D absorption impaired includes celiac disease, biliary obstruction, chronic pancreatitis, liver failure, cystic fibrosis, Crohn’s disease, and gastric bypass. Individuals on bile acid-binding medications such as colestyramine and colestipol have impaired vitamin D absorption (42).
2.14.9 Obesity
There are inverse correlation between vitamin D level and adipose tissue. Several studies have shown that obese individuals have lower serum concentrations of 25(OH)D$_3$ than those with normal weights (213).

2.14.10 Metabolism of vitamin D
Catabolism of 25(OH)D and 1,25(OH)$_2$D is primarily mediated by CYP24 [25(OH)D-24 - hydroxylase] in the kidney and by CYP3A4 in the liver and small intestine (214). The long-term use of certain medications such as phenobarbital, phenytoin, carbamazepine, rifampicin and antiretrovirals (HAART), causes up regulation of CYP3A4 and leads to decreased levels of 25(OH)D and 1,25(OH)$_2$D (42).

C. Diseases
2.14.11 Kidney disease
A loss of kidney function leads to a decline in circulating 1,25(OH)$_2$D levels. Low levels of 25(OH)D can occur in nephrotic-range proteinuria due to direct loss of vitamin DBP-bound 25(OH)D in the urine (215).

2.14.12 Liver disease
The decrease level of 25(OH)D occur in hepatobiliary disease, cholestatic liver disease and parenchymal liver disease. Impaired synthesis of vitamin DBP observed in fulminant hepatic failure and chronic liver disease (216).
TABLE 2.7: Risk factor for Vitamin D availability/synthesis

| 1. UVB radiation |
| 2. Latitude |
| 3. Clothing |
| 4. Use of Sunscreen |
| 5. Melanin |
| 6. Age |
| 7. Skin temperature |
| 8. Obesity |
| 9. Malabsorption |
| 10. Kidney disease |
| 11. Liver disease |
| 12. Drugs like phenobarbital, phenytoin, carbamazepine, rifampicin and antiretrovirals |

2.15 Vitamin D Deficiency and Insufficiency

Serum 25(OH)D is the best indicator for overall vitamin D status in the body which reflects total vitamin D from sun exposure, dietary intake and internal conversion of vitamin D from adipose tissue. Vitamin D deficiency is defined as a 25(OH)D level of less than 20 ng/ml (50 nmol/L) (42). The 25(OH)D levels are inversely related to parathyroid hormone levels until the former reach 30–40 ng/ml (75 to 100 nmol/L) (42). Intestinal calcium transport increases by 45 to 65% when 25(OH)D levels increase from an average of 20 to 32 ng/ml (50 to 80 nmol/L) (13,72). Vitamin D insufficiency has been defined as a 25(OH)D of 21–29 ng/ml (52 to 72 nmol per liter) (42). The 25(OH)D levels of 30-40 ng/ml (75 to 100 nmol/L) are indicative of normal vitamin D levels. Vitamin D intoxication is rare and occur when serum levels of 25-hydroxyvitamin D are greater than 150 ng per milliliter (374 nmol per liter) (42).
TABLE 2.8: Vitamin D Status

<table>
<thead>
<tr>
<th>Health Status</th>
<th>Serum 25-hydroxyvitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/ml</td>
</tr>
<tr>
<td>Deficiency</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Insufficiency</td>
<td>21–29</td>
</tr>
<tr>
<td>Sufficiency</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Intoxication</td>
<td>&gt;150</td>
</tr>
</tbody>
</table>

2.16 Prevalence of Vitamin D Deficiency and Insufficiency

Vitamin D deficiency is the most common medical conditions and it has been estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency (42). It has been estimated that 20–100% of U.S., Canadian, and European elderly men and women are vitamin D deficient. Vitamin D deficiency is common in Australia, the Middle East, India, Africa, and South America (42). The worldwide status of vitamin D is shown in table 3 (217). Pregnant and lactating women who take a prenatal vitamin and a calcium supplement with vitamin D remain at high risk for vitamin D deficiency (218).

FIGURE 2.17: Percent of population with less than 20 ng/ml of vitamin D
2.17 Dosing and safety of Vitamin D

Only a few foods are a good source of vitamin D which includes fatty fish, beef liver, and egg yolks. Multivitamins contain about 400 to 1,000 IU of vitamin D. A variety of options are available for vitamin D supplements which including sachet, capsules, chewable tablets, liquids, and drops (219). In general, 100 IU of vitamin D daily can raise blood concentrations 1 ng/mL after 2 to 3 months (Tab. 2.9) (220). Once the desired blood concentration is achieved it can be maintain with daily dosing of 800 to 1,000 IU of vitamin D (220).

**TABLE 2.9: Dosing and Blood Concentrations (30)**

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Expected Increase in Blood Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 IU</td>
<td>1 ng/mL</td>
</tr>
<tr>
<td>200 IU</td>
<td>2 ng/mL</td>
</tr>
<tr>
<td>400 IU</td>
<td>4 ng/mL</td>
</tr>
<tr>
<td>800 IU</td>
<td>8 ng/mL</td>
</tr>
<tr>
<td>1,000 IU</td>
<td>10 ng/mL</td>
</tr>
<tr>
<td>2,000 IU</td>
<td>20 ng/mL</td>
</tr>
</tbody>
</table>

A variety of alternative dosing regimens have been published over the past decade. A high-dose of short-course regimen 100,000 to 600,000 IU (2.5 to 15 mg) over 1 to 5 days has been suggested for patients who might not adhere to longer regimens (221). Ilahi et al. (222) performed pharmacokinetics of a single, large dose of cholecalciferol. In that study one group (n=30) was supplemented with a single oral dose of 100 000 IU cholecalciferol and second group (n=10) served as a control group to assess the seasonal change of calcidiol. Serum calcidiol concentrations were followed for 4 month. They found that serum calcidiol rose promptly after cholecalciferol dosing from a mean (±SD) baseline of 27.1±7.7 ng/mL to a concentration maximum of 42.0±9.1 ng/mL (Figure 2.18). The mean Cmax rise from baseline was 14.9 ±5.1 ng/mL. The peak occurred at 7 day (median Tmax), and the serum concentration declined approximately linearly thereafter. Mean values no longer significantly different from baseline were reached by 84 day, and the mean calcidiol concentration also fell below 32.1 ng/mL by 84 days. However, control group had a nonsignificant change from baseline of -0.72 ± 0.80ng/mL during 4 month. Therefore, 100 000 IU cholecalciferol is a safe, efficient, and cost-effective means to increase calcidiol
concentrations in the elderly. From this study one can safely recommend 100,000 IU cholecalciferol dosed every 2 months in persons with moderate baseline calcidiol concentrations. However, in those persons with baseline calcidiol concentrations 20 ng/mL, even this large dose will not adequately raise their calcidiol concentrations.

FIGURE 2.18: Time course of serum calcidiol for 112 d (16 wk) after a single oral dose of 100,000 IU cholecalciferol (n 30). The error bars are 1 SEM. The horizontal dashed line demarcates values above and below 80 nmol/L.

The larger intakes of vitamin D also tested. Administration of vitamin D up to 80,000 IU daily for 1 year has serum 25-OHD concentrations up to about 1250 nmol/L, without developing hypercalcemia or other signs of vitamin D toxicity (223) while in another shorter study, the consumption of 100,000 IU of vitamin D daily for 4 days did not affect serum calcium or phosphorus concentrations and the greatest serum 25-OHD concentration was lower than 250 nmol/L (224). In another short-term study, the daily consumption of 160,000 IU of vitamin D for 7 days produced serum 25-OHD concentrations of up to nearly 600 nmol/L without producing adverse effects or hypercalcemia (223). Various large amounts of oral vitamin D have been studied in single-bolus acute studies. In one experiment, the consumption of a single bolus of 30,000 IU of vitamin D was followed by maximum serum 25-OHD concentrations of up to 175 nmol/L without adverse reactions or side effects (225).
In another experiment, the consumption of a single bolus of 50,000 IU of vitamin D was followed by maximum serum 25-OHD concentrations of up to 173 nmol/L without adverse reactions or side effects (226). In a double-blind, randomized, placebo-controlled clinical trial, relatively healthy, independently living elderly men and women initially 65 to 85 years old consumed a placebo or 100,000 IU of vitamin D once every 4 months for 5 years. There were no adverse reactions or side effects attributable to vitamin supplementation. Therefore, high dose of vitamin D are very safe and does not cause any adverse effect unless and until serum 25(OH)D >150 ng/ml and that adverse effect can be diminished after discontinuation of vitamin D treatment.

2.18 Adverse Effects of Vitamin D
Excessive administration of vitamin D may result in weakness, headache, somnolence, nausea, vomiting, dry mouth, a metallic taste, constipation, muscle or bone pain. With continued high-dose vitamin D intake (> 10,000 International Units/day) or overdose (serum 25(OH)D levels > 150 ng/mL), patients may develop polyuria, polydipsia, anorexia, irritability, mild acidosis, hypercalciuria, anemia, azotemia, nephrocalcinosis, vascular calcifications, pancreatitis, photophobia, rhinorrhea, pruritus, hyperthermia, cardiovascular instability, bone demineralization, and hepatic or renal dysfunction. These symptoms may persist for several months after discontinuing therapy (227).

The main goal of treatment is correction of the hypercalcemia. When the calcium concentration exceeds 14 mg/dl, emergency intervention is necessary because of the adverse effects of hypercalcemia on cardiac, central nervous system, renal, and gastrointestinal functions. Treatment for vitamin D toxicity includes: discontinuing intake, a diet with low calcium and phosphorus content, intravenous (IV) hydration, loop diuretics, glucocorticoids, and calcitonin. More recently, oral and IV bisphosphonates have been proven to be effective in the treatment of vitamin D intoxication while IV hydration and diuretics are used for mild cases (227).

2.19 Vitamin D receptor binding and activation
The biological responses to the 1,25(OH)2D3 are almost entirely mediated by a nuclear vitamin D receptor (VDR). VDR is a DNA-binding transcription factor; encoded by the VDR gene (228). The binding of 1,25(OH)2D3 to VDR initiate interaction with the retinoid X receptor (RXR) to form heterodimer. This heterodimer binds to the VDR response element
(VDRE) and initiates recruitment of nuclear proteins into the transcriptional complex (229). Binding of 1,25(OH)2D3 is thought to bring about a conformational change in the receptor structure that increases interactions with DNA (230) and distinct nuclear proteins (231). Overall activated VDR leads to exertion of most of the 1,25(OH)2D3 functions.

### 2.20 Immune Effects of Vitamin D

The recent data has shown that vitamin D has direct effects on activated helper T cells, regulatory T cells, activated B cells and dendritic cells (232). Vitamin D inhibits the polarization of naive Th0 cells to Th1, Th2 cells and up regulates Treg cells which known to inhibits both Th1 and Th2 cells (233). However, there are conflicting evidences, both enhancement and inhibition for effects of vitamin D on Th2 responses (234). Treatment with vitamin D has shown reduced level of IL-4 concentrations in bronchoalveolar lavage fluid, and an attenuated Th2 dependent inflammatory response in vivo (235). Moreover, vitamin D reduces the production and expression of the Th1 associated cytokines such as IL-2, TNF-α, and IFNγ (236). Taher et al., has shown beneficial effect of vitamin D treatments to post allergic sensitization mice (237) while Wittke et al., had shown no effect on the severity of allergic airway disease induced by OVA (238). Human studies also suggested that vitamin D deficiency causes impaired lung function (239). In asthma, reduced vitamin D levels are associated with impaired lung function, increased airway hyperresponsiveness, reduced glucocorticoid response (49) and increased expression of TNF-α (48). However, there are some reports shown non-linear response to vitamin D in that in that both high and low levels have been associated with increased Th2 activity. Vitamin D inhibited both Th1 and Th2 cytokines production at low concentration but failed to inhibit at high concentrations (240). Hypponen et al., (75) demonstrated a U-shaped relationship between serum 25(OH)D and IgE concentrations in adults with both very high (>130 ng/ml) and very low (<10 ng/ml) serum 25(OH)D associated with higher levels of IgE (Fig 2.19).
FIGURE 2.19: Variation in the average immunoglobulin E by 25-hydroxyvitamin D concentration in the 1958 British cohort at the age of 45 years. Data are season and sex-adjusted geometric mean values, 95% confidence intervals given in error bars.

TABLE 2.10: Response of various inflammatory cell to Vitamin D

<table>
<thead>
<tr>
<th>Target Cell</th>
<th>Effect of Vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1</td>
<td>Inhibit Th1 associated cytokine release</td>
</tr>
<tr>
<td>Th2</td>
<td>Conflicting evidences, both enhancement and inhibition</td>
</tr>
<tr>
<td>Th17</td>
<td>Inhibit Th17 associated cytokine release</td>
</tr>
<tr>
<td>Treg</td>
<td>Induces IL-10 synthesis</td>
</tr>
<tr>
<td>B-cells</td>
<td>Enhance synthesis of IL-10</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>Induces IL-10 synthesis, decreased DC maturation</td>
</tr>
<tr>
<td>Matrix metalloproteinases-9</td>
<td>Attenuates production by inhibiting signalling cascades, c-Jun-N-terminal kinase (JNK) and nuclear factor kB (NFkB)</td>
</tr>
</tbody>
</table>
FIGURE 2.20: Immune effects of vitamin D (calcitriol)

Recently, 1,25(OH)2D3 inhibit Th17 associated cytokines in vitro and in vivo in experiments which may be important in steroid refractory airway disease (51). Th17 produced more inflammatory cytokines such as IL-1β, IL-6 and IL-17 which plays a mechanistic role in increasing asthma severity and reducing corticosteroid sensitivity (53). Vitamin D inhibits maturation of monocyte-derived DCs, induces Tregs cells proliferation
(241, 242) and increases IL-10-secretion and toll-like receptor (TLR)-9 expression (56, 57). Moreover, treatment with vitamin D to glucocorticoid-resistant asthmatic patients enhanced the response to dexamethasone by restoring the defective IL-10 (57). In vitro study, glucocorticoids induced dose dependent synthesis of IL-10 via stimulation of CD4+ T cells. Even most of the steroid resistant asthmatics fail to increase IL-10 synthesis from CD4+ T cells in the presence of the glucocorticoids (243) and this steroid-induced IL-10 synthesis can be enhance by the addition of vitamin D to the T cell culture. In a small a pilot study, administration of vitamin D to steroid resistant asthmatic patients enhanced their response to dexamethasone for the induction of IL-10 (57). In another in vitro study, addition of vitamin D enhanced glucocorticoid action in peripheral blood mononuclear cells (PBMCs) from asthmatic patients and decreased the active dose of dexamethasone greater than 10-fold (31). Searing et al., showed significant associations between inhaled corticosteroids use, oral corticosteroids use, and total steroid dose with lower vitamin D levels (31). All this studies suggested that supplementation of vitamin D could potentially increase the therapeutic response to glucocorticoids in steroid-resistant asthmatics patients and potentiate the anti-inflammatory function of corticosteroids in asthmatic patients.

2.21 Epidemiological evidence of a link between vitamin D levels and asthma

Wu et al., (67) assessed the effect of vitamin D levels on pre-bronchodilator forced expiratory volume in 1 second (FEV1), bronchodilator response (BDR), and responsiveness to methacholine (PC20) in asthmatics treated with inhaled corticosteroids. In the inhaled corticosteroid treatment group, pre-bronchodilator FEV1 increased from randomization to 12 months by 140 ml in the vitamin D deficient group while prebronchodilator FEV1 increased by 330 ml in the vitamin D insufficiency group and 290 ml in the vitamin D sufficiency group (p=0.0072), in adjusted models. The study concluded that vitamin D deficiency is associated with poorer lung function than children with vitamin D insufficiency or sufficiency (67). A cross-sectional study of 560 children ages 6-14 years with (n =287) and without (n = 273) asthma in San Juan, Puerto Rico showed vitamin D insufficiency was associated with severe asthma exacerbations (68). A decreased level of vitamin D associated with significantly increased odds of asthmatic state (P=0.002) (69). In asthmatic patients, 25-hydroxy vitamin D levels had direct and significant correlations with both predicted FEV1 (Fig 2.21) and FEV1/FVC (31, 70-72). The 25(OH)D(3) levels inversely associated with exacerbations (r = -0.6, P < 0.001) and inhaled steroid dose (r = -0.39, P = 0.001) in severe therapy-resistant asthma and moderate asthma. Airway smooth muscle mass was
inversely related to 25(OH)D(3) levels (r = -0.6, P = 0.008) (70). Therefore, higher vitamin D levels were associated with greater lung function and increase in FEV(1) for each nanogram per milliliter increase in vitamin D (P = 0.02) (244).

**FIGURE 2.21.** Plot of serum vitamin D (25[OH]D, ng/ml) versus prebronchodilator FEV1 (L), representing an increase of 22.7 (69.3) ml in FEV1 for each nanogram per milliliter increase in vitamin D (P 5 0.02;covariate adjusted r 5 0.8).

A randomized clinical trial of 130 individuals with aged 10 to 50 years showed that 24 weeks of vitamin D supplementation significantly improve FEV1 in mild to moderate persistent asthma (245). In similar way another study of 100 asthmatic children of either sex received oral vitamin D3 60,000 IU per month for 6 months and the other group received placebo. Monthly doses of 60,000 IU vitamin D significantly reduced the number of exacerbations as compared to placebo (p = 0.011). PEFR significantly increased in the treatment group (p = 0.000). Monthly doses of vitamin D significantly reduced the requirement of steroids (p = 0.013) and emergency visits (p = 0.015). Control of asthma was achieved earlier in patients who received monthly vitamin D. Vitamin D significantly reduced the level of severity of asthma patients over 6 months of treatment (p = 0.016). Vitamin D has a definite role in the management of moderate to severe persistent bronchial asthma as an adjunct to standard treatment (246).
Searing et al., observed a significant inverse correlations with vitamin D levels and use of inhaled steroids (P = .0475), use of oral steroids (P = .02), and total steroid dose (P = .001). In an experimental model of steroid resistance in which dexamethasone alone did not inhibit T-cell proliferation but addition of vitamin D to dexamethasone resulted in significant dose-dependent suppression of cell proliferation. Therefore, corticosteroid use and worsening airflow limitation are associated with lower vitamin D serum levels in asthmatic patients. In vitro study; Vitamin D enhances glucocorticoid action in PBMCs from asthmatic patients and enhances the immunosuppressive function of dexamethasone (31). Vitamin D levels were significantly and inversely associated with total IgE, allergic disease and eosinophil count. Therefore; the increased serum 25-(OH)D(3) level may inhibit total IgE expression, suggesting that increasing serum 25-(OH)D(3) level might be a new option for the prevention and treatment of asthma (73, 74, 247-250).

In a case control study of thirty-nine children with clinically controlled asthma; there was a significant correlation of 25(OH) D level with Th1/Th2 ratio (Fig 2.22), (r =0.698; P = 0.0001), CD25(+)Foxp3(+) Treg cells and significant negative correlation with interleukin-17 (Fig 2.23), (r = -0.617; P = 0.001). These findings suggest that vitamin D is an important promoter of T cell regulation in vivo in young asthmatics (251). In a multicenter clinical trial; 1024 children with mild-to-moderate persistent asthma at the time of enrollment were randomized to receive budesonide, nedocromil, or placebo. Vitamin D insufficiency was common in children with mild-to-moderate persistent asthma and is associated with higher odds of severe exacerbation over a 4-year period (252). Third National Health and Nutrition Examination Survey, examined the association between 25(OH)D level and recent upper respiratory tract infections in 18 883 participants 12 years and older. Serum 25(OH)D levels are inversely associated with recent upper respiratory tract infections. This association may be stronger in those with respiratory tract diseases (253).
FIGURE 2.22: Correlation between Th1/Th2 ratio and vitamin D levels in asthmatic patients using Pearson’s correlation coefficient.

FIGURE 2.23: Correlation between vitamin D level and serum IL-17

A cross-sectional study of 1745 pregnant women showed vitamin D intake positively related to the prevalence of asthma in young adult Japanese women (254). Another cross-sectional analyses of 340 mother-infant dyads demonstrated that higher maternal vitamin D levels
were associated with decreased odds of asthma (255). Children (n=1669) participating in the population-based birth cohort study were followed for asthma, allergic rhinitis (AR) and atopic eczema assessed by validated questionnaire at 5 years (256). The maternal intake of vitamin D from food was negatively related to risk of asthma [hazard ratio (HR) 0.80; 95% confidence interval (CI) 0.64-0.99] and AR [HR 0.85; 95% CI 0.75-0.97]. Therefore, maternal vitamin D intake from foods during pregnancy may be negatively associated with risk of asthma and AR in childhood. A decreased bronchodilator response (P = 0.04) were associated with lower maternal total vitamin D intakes in pregnancy (257). Therefore, increase of maternal vitamin D intakes during pregnancy may decrease the risk of wheeze symptoms in early childhood (258). In a double-blind, single-center, randomized clinical trial of 623 women at 24 weeks of pregnancy supplemented with vitamin D3 (2400 IU/d; n = 315) or matching placebo tablets (n = 308) from pregnancy week 24 to 1 week postpartum and follow-up of the children (N = 581) was completed when the youngest child reached age 3 years. Of the 581 children, persistent wheeze was diagnosed during the first 3 years of life in 47 children (16%) in the vitamin D3 group and 57 children (20%) in the control group. Vitamin D3 supplementation was not associated with the risk of persistent wheeze, but the number of episodes of troublesome lung symptoms was reduced, and the airway immune profile was up-regulated (principal component analysis, P = .04) (259).

**TABLE 2.11:** Epidemiological studies of vitamin D levels and asthma

<table>
<thead>
<tr>
<th>Investigator (Reference)</th>
<th>Study Type</th>
<th>No. of subjects</th>
<th>Age</th>
<th>Final outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yadav et al., 2014 (246)</td>
<td>Randomized trial</td>
<td>100 children</td>
<td></td>
<td>Vitamin D supplementation significantly reduced the level of severity of asthma</td>
</tr>
<tr>
<td>Arshi et al., 2014 (245)</td>
<td>Randomized trial</td>
<td>130 children and adults</td>
<td>10-50 years</td>
<td>Vitamin D supplementation significantly improve FEV1 in mild to moderate persistent asthma</td>
</tr>
<tr>
<td>Wu et al., 2012 (67)</td>
<td>Randomized trial</td>
<td>1024 children</td>
<td>5-12 years</td>
<td>Vitamin D deficiency associated with poorer lung function</td>
</tr>
<tr>
<td>Brehm et al., 2012 (68)</td>
<td>Cross-sectional study</td>
<td>560 children</td>
<td>6-14 years</td>
<td>Vitamin D insufficiency associated with severe asthma exacerbations</td>
</tr>
<tr>
<td>Author et al., Year (Page)</td>
<td>Study Design</td>
<td>Participants</td>
<td>Age Range</td>
<td>Findings</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------</td>
<td>--------------</td>
<td>-----------</td>
<td>----------</td>
</tr>
<tr>
<td>Alyasin et al., 2011 (69)</td>
<td>Cross-sectional study</td>
<td>100 children</td>
<td>6-18 years</td>
<td>Vitamin D levels inversely associated with asthma</td>
</tr>
<tr>
<td>Gupta et al., 2011 (70)</td>
<td></td>
<td>86 children</td>
<td>mean age, 11.7 yr</td>
<td>Airway smooth muscle inversely related to 25(OH)D(3) levels</td>
</tr>
<tr>
<td>Li et al., 2011 (71)</td>
<td>Cross-sectional study</td>
<td>435 patients</td>
<td>&gt;18 years</td>
<td>Vitamin D deficiency highly prevalent in asthmatic patients and vitamin D status was associated with lung function.</td>
</tr>
<tr>
<td>Chinellato et al., 2011 (72)</td>
<td>Cross-sectional study</td>
<td>75 children</td>
<td>5-11 years</td>
<td>Hypovitaminosis D is frequent in children with asthma and lower levels of vitamin D are associated with reduced asthma control.</td>
</tr>
<tr>
<td>Searing et al., 2010 (31)</td>
<td></td>
<td>100 children</td>
<td>0-18 years</td>
<td>VitD enhances glucocorticoid action in PBMCs and immunosuppressive function</td>
</tr>
<tr>
<td>Sutherland et al., 2010 (244)</td>
<td></td>
<td>54 adults</td>
<td>&gt;18 years</td>
<td>Reduced vitamin D levels are associated with impaired lung function, increased AHR, and reduced GC response</td>
</tr>
<tr>
<td>Brehm et al., 2009 (73)</td>
<td>Cross-sectional study</td>
<td>616 children</td>
<td>6-14 years</td>
<td>Low level of vitamin D associated with increased markers of allergy and asthma severity</td>
</tr>
<tr>
<td>Goleva et al., 2012 (247)</td>
<td>Prospective study</td>
<td>205 adults and children</td>
<td>Serum vitamin D level had significant inverse relationship with daily inhaled corticosteroid dose</td>
<td></td>
</tr>
<tr>
<td>Bener et al., 2012 (248)</td>
<td></td>
<td>966 children</td>
<td>&lt;16 years</td>
<td>Asthmatic children had vitamin D deficiency</td>
</tr>
<tr>
<td>Ehlayel et al., 2011 (249)</td>
<td>Case-control study</td>
<td>483 cases</td>
<td>&lt; 15 years</td>
<td>Lower Vitamin D levels associated with more allergic disease and elevated serum IgE</td>
</tr>
<tr>
<td>Ma et al., 2011 (74)</td>
<td></td>
<td>110 children</td>
<td></td>
<td>increased serum 25-(OH)D(3) level may inhibit total IgE expression</td>
</tr>
<tr>
<td>Maalmi et al., 2012 (251)</td>
<td>Case-control study</td>
<td>39 children</td>
<td>6-16 years</td>
<td>Vitamin D promote of T cell regulation</td>
</tr>
<tr>
<td>Study</td>
<td>Type/Study Design</td>
<td>Participants</td>
<td>Age Range</td>
<td>Findings</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------------------</td>
<td>--------------</td>
<td>-----------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Brehm et al., 2010</td>
<td>Multicenter clinical trial</td>
<td>1024 children</td>
<td>7.2 – 10.6 years</td>
<td>Vitamin D insufficiency common in children with mild-to-moderate persistent asthma</td>
</tr>
<tr>
<td>Ginde et al., 2009</td>
<td>Third National Health and Nutrition Examination Survey</td>
<td>18 883 participants</td>
<td>&gt;12 years</td>
<td>Serum 25(OH)D levels are inversely associated with recent URTI</td>
</tr>
<tr>
<td>Miyake et al., 2012</td>
<td>Cross-sectional study</td>
<td>1745 pregnant women</td>
<td></td>
<td>Vitamin D intake positively related to the prevalence of asthma</td>
</tr>
<tr>
<td>Carroll et al., 2011</td>
<td>Cross-sectional study</td>
<td>340 mother-infant dyads</td>
<td></td>
<td>Higher maternal vitamin D levels associated with decreased odds of asthma.</td>
</tr>
<tr>
<td>Erkkola et al., 2009</td>
<td>Birth cohort study</td>
<td>1669 children</td>
<td></td>
<td>Maternal vitamin D intake from foods during pregnancy may be negatively associated with risk of asthma and AR in childhood.</td>
</tr>
<tr>
<td>Devereux et al., 2007</td>
<td>Birth cohort study</td>
<td>2000 healthy pregnant</td>
<td></td>
<td>Increased maternal vitamin D intakes during pregnancy decreased risk of wheeze symptoms in early childhood</td>
</tr>
<tr>
<td>Camargo et al., 2007</td>
<td>Prospective prebirth cohort study</td>
<td>1194 mother-child pairs</td>
<td></td>
<td>Higher maternal intake of vitamin D during pregnancy decreased the risk of recurrent wheeze in early childhood</td>
</tr>
<tr>
<td>Chawes et al., 2016</td>
<td>Randomized trial</td>
<td>623 women at 24 weeks of pregnancy</td>
<td></td>
<td>Vitamin D3 supplementation reduces the number of episodes of troublesome lung symptoms and up regulate airway immune profile</td>
</tr>
</tbody>
</table>
2.21 Other Health effects of Vitamin D

2.21.1 Diabetes

There is growing evidence that vitamin D deficiency could be a contributing factor in the development of both type 1 and type 2 diabetes. The β-cell in the pancreas contains VDRs as well as the 1 alpha hydroxylase enzyme (260). Evidence indicates that vitamin D treatment improves glucose tolerance and insulin resistance (261) while vitamin D deficiency reduced insulin secretion. Supplementation with vitamin D has been shown to restore insulin secretion in animals (262). In studies of nonobese diabetic mice, high doses of vitamin D have been shown to delay the onset of diabetes (263) by protecting beta cell function caused by inflammatory cytokines (IL-6 and TNF-alpha) (264). Recent evidence has demonstrated that persons with type 2 diabetes who have hypovitaminosis D are more likely to have increased CRP (P = .001), fibrinogen (P = .001), and HbA1c (P = .01) compared with those persons with diabetes who do not (265). There is strong evidence that activation of inflammatory pathways interferes with normal metabolism and disrupts proper insulin signaling (266).

Study observed that there was a 60% increase in insulin sensitivity in individuals with serum concentrations of 25(OH)D of 30 ng/mL compared to 10 ng/mL and concentrations <20 ng/mL were associated with decreased beta-cell function (267). The NHANES III study found that individuals with a 25(OH)D concentration of <21 ng/mL doubled their odds ratio for diabetes, compared to those with concentrations above 37 ng/mL (268). A study of >10,000 Finnish children who were given 2,000 IU vitamin D3 per day during the first year of life demonstrated a 78% reduced risk of type 1 diabetes over a 30-year follow-up (269). Subsequently, this finding was confirmed by a meta-analysis of 5 observational studies in England (270). One study reported that a daily intake of >800 IU of vitamin D compared with <400 IU of vitamin D reduced the risk of T2DM by nearly one-third (271). In a 6-month study of 81 South Asian women with insulin resistance, 1,000 IU daily of vitamin D reduced insulin resistance and improved insulin sensitivity, reducing fasting insulin levels without changing insulin secretion (261). However, in review by Pittas et al. (272) of 13 observational studies (14 cohorts) and 18 trials, 3 of 6 analyzed from 4 different cohorts, reported a lower incident T2DM in the highest versus the lowest vitamin D status group, and 8 trials found no effect of vitamin D supplementation on glycaemia or incident T2DM. Although the reasons for these inconsistencies in both the hypertension and T2DM trials are uncertain, potential explanations include baseline 25(OH)D levels, various vitamin D doses,
achieved 25(OH)D levels, and various study designs (including primary, secondary, and tertiary prevention trials).

### 2.21.2 Serum lipids

Activation of VDR results in decreased accumulation of neutral lipids (triglycerides and cholesterol). A decrease in triglycerides is mediated by vitamin D-induced 1) decrease in fatty acid synthesis as a result of decreases in sterol regulatory element binding proteins (SREBP)-1 which are the major mediators of fatty acid and triglyceride synthesis 2) increase in fatty acid oxidation as a result of increased PPAR-α, and 3) decrease in fatty acid uptake as a result of decrease in CD36. The decrease in cholesterol is mediated by vitamin D-induced 1) decrease in cholesterol synthesis as a result of decreases in SREBP-2 and HMG CoA reductase and 2) decrease in cholesterol uptake as a result of a decrease in LDL receptor (273).

A study by Jorde et al. (274) who included 8018 nonsmoking subjects in the cross-sectional study, found significant positive associations between serum 25(OH)D and serum TC, HDL-C and LDL-C, and significant negative associations between serum 25(OH)D and both LDL-C/HDLC ratio and TG after adjustment for gender, age, BMI and month of blood sampling. On the other hand, all studies report a positive association between high-density lipoprotein cholesterol (HDL-C) and 25(OH)D, and five out of eight reported a positive association with low-density lipoprotein cholesterol (LDL-C). The negative associating between 25(OH)D and TG was also reported from a longitudinal study in that 1762 subjects were followed for 14 years showed increase in serum 25(OH)D results in significant decrease in serum TG (274).

### 2.21.3 Hypertension

The renin-angiotensin-aldosterone system (RAAS) plays a major role in the pathogenesis of cardiovascular diseases (275). Vitamin D helps in the regulation of blood pressure and hormonal mechanisms regulating in blood pressure (276). Observational reports have shown higher blood pressure trends in winter months and locations further from the equator, suggesting that low ultraviolet radiation and thus decreased capacity for cutaneous vitamin D synthesis are associated with hypertension (210). Vitamin D is a negative regulator of the RAAS and also influences vascular endothelial function (277) or vascular smooth muscle intracellular calcium concentrations (278). Li et al (277) showed that VDR knockout (KO)
mice had significant elevations in renin activity and circulating plasma angiotensin II concentrations. These mice developed hypertension and cardiac hypertrophy that could be attenuated with the administration of RAAS antagonist pharmacotherapy, and also exhibited increased activity of the local cardiac tissue RAAS (277).

A retrospective observational studies have shown significant inverse correlations between vitamin D levels and systolic blood pressure (279). In a small study from Belgium of 25 patients with hypertension, vitamin D levels were inversely correlated with systolic blood pressure, diastolic blood pressure, and calf vascular resistance (279). In a subsequent study involving normotensive men, a similar inverse correlation between 1,25(OH)2 vitamin D and systolic blood pressure was observed (280). In a randomized study, women aged >70 years with 25(OH) vitamin D levels 20 ng/ml were randomly assigned to receive supplementation with calcium 1,200 mg/day or calcium 1,200 mg/day plus vitamin D (cholecalciferol) 800 IU/day. Within 8 weeks of treatment, systolic blood pressure in the vitamin D–treated group had decreased by an average of 13 mm of Hg (p=0.02) (281, 282). In a similar randomized study involving patients with diabetes mellitus and serum 25(OH) vitamin D levels 20 ng/ml, patients were randomly assigned to receive a 1-time dose of ergocalciferol 100,000 IU or placebo. Vitamin D supplementation produced a significant decrease in systolic blood pressure (282, 283)

### 2.21.4 Kidney disease

Observational studies have shown associations between lower 25(OH)D concentrations and the prevalence of CKD and proteinuria (284). A systematic review and meta-analysis of 22 studies (17 observational and 5 randomized trials) of vitamin D2 or D3 supplementation suggested that, although these interventions expectedly raised 25(OH)D and lowered parathyroid hormone levels in CKD, they did not significantly affect other biochemical markers of kidney disease progression (285) Ravani et al (286) observed an inverse and independent longitudinal relationship between 25(OH)D concentrations and the progression of kidney disease in 168 individuals with non–dialysis-dependent CKD (stages 2-5) over 2 years. Agarwal et al (287) aggregately evaluated 220 patients with CKD (stages 3 or 4) from 3 randomized placebo-controlled studies evaluating the effect of paracalcitrol therapy vs placebo for up to 6 months. Half of the proteinuric subjects receiving paracalcitrol experienced reductions in automated dipstick proteinuria, in comparison to only a quarter of those receiving placebo (P < .01).
In a study of 825 consecutive U.S. hemodialysis patients, 78% were found to have vitamin D deficiency and 18% were considered to be severely deficient (288). In a large study of >51,000 end-stage CKD patients who survived for at least 90 days after initiation of hemodialysis, 2-year survival was assessed. Analyzing 13,864 who died and 37,173 who survived, 2-year mortality was more than doubled among those who did not receive injectable vitamin D therapy compared to those who did. In multivariate analysis, injectable vitamin D therapy was associated with an independent 20% reduction in overall mortality (289). Other observational studies of patients with CKD and hyperparathyroidism found that oral administration of 1,25(OH)2D3 (also known as activated vitamin D or calcitriol) is associated with improved survival (290). Thus far, vitamin D analogs given to hemodialysis patients have been shown to improve survival.

### 2.21.5 Depression

Recently, vitamin D has been implicated as a factor affecting cognitive function and mental health and vitamin D concentrations have been found to be low in patients with mood disorders, including depression (291). In data from the NHANES III study, in nearly 8,000 no institutionalized U.S. residents, the likelihood of having depression was significantly higher among those with vitamin D deficiency (291). Likewise, among a CV disease population of >7,000 patients ≥50 years of age with no history of depression, low vitamin D levels were shown to be an associated with incident depression (292). It is known that vitamin D receptors are present in the brain, and enzymes are present in the central nervous system that are involved in vitamin D hydroxylation (293). Additionally, elevated levels of PTH due to vitamin D deficiency may also play a role in the development of depression (294). Recently, a dose of 100,000 IU of vitamin D was found superior to light therapy in the treatment of seasonal affective disorder after one month (295). Similarly, in a study involving 44 subjects, supplementation with 400 or 800 IU per day was found to significantly improve mood within five days of supplementation (296).

### 2.21.6 Epilepsy

Seizures can be the presenting manifestation of vitamin D deficiency. Hypovitaminosis D decreases the threshold for and increases the incidence of seizures, and several “anticonvulsant” drugs interfere with the formation of calcitriol in the kidney and further reduce calcitriol levels via induction of hepatic clearance. Therefore, antiepileptic drugs may lead to iatrogenic seizures by causing iatrogenic hypovitaminosis D (297). Conversely,
supplementation with 4,000–16,000 IU per day of vitamin D2 was shown to significantly reduce seizure frequency in a placebo controlled pilot study by Christiansen et al (298).

### 2.21.7 Migraine Headaches
Calcium clearly plays a role in the maintenance of vascular tone and coagulation, both of which are altered in patients with migraine. Thys-Jacobs reported two cases showing a reduction in frequency, duration, and severity of menstrual migraine attacks following daily supplementation with 1,200 mg of calcium and 1,200–1,600 IU of vitamin D in women with vitamin D deficiency (299).

### 2.21.8 Cancer Prevention
Vitamin D has anti-cancer effects mediated by anti-proliferative and proapoptotic mechanisms which are augmented by modulation of nuclear receptor function and enzyme action and limited research shows that synthetic vitamin D analogs may have a role in the treatment of human cancers (300). Grant (301) has shown that inadequate exposure to sunlight, and hence hypovitaminosis D, is associated with an increased risk of cancer mortality for several malignancies, namely those of the breast, colon, ovary, prostate, bladder, esophagus, kidney, lung, pancreas, rectum, stomach, uterus, and non-Hodgkin lymphoma. He proposes that adequate exposure to ultraviolet light and/or supplementation with vitamin D could save more than 23,000 American lives per year from a reduction in cancer mortality alone. Both prospective and retrospective epidemiologic studies indicate that levels of 25-hydroxyvitamin D below 20 ng per milliliter are associated with a 30 to 50% increased risk of incident colon, prostate, and breast cancer, along with higher mortality from these cancers (302). A prospective study of vitamin D intake and the risk of colorectal cancer in 1954 men showed a direct relationship (with a relative risk of 1.0 when vitamin D intake was 6 to 94 IU per day and a relative risk of 0.53 when the intake was 233 to 652 IU per day, P<0.05) (303). Participants in the Women's Health Initiative who at baseline had a 25-hydroxyvitamin D concentration of less than 12 ng per milliliter (30 nmol per liter) had a 253% increase in the risk of colorectal cancer over a follow-up period of 8 years (304). In a study of men with prostate cancer, the disease developed 3 to 5 years later in the men who worked outdoors than in those who worked indoors (305). Pooled data for 980 women showed that the highest vitamin D intake, as compared with the lowest, correlated with a 50% lower risk of breast cancer (302).
2.21.9 Osteoporosis and Fracture

Approximately 33% of women 60 to 70 years of age and 66% of those 80 years of age or older have osteoporosis (306). It is estimated that 47% of women and 22% of men 50 years of age or older will sustain an osteoporotic fracture in their remaining lifetime. Chapuy et al. (307) reported that among 3270 elderly French women given 1200 mg of calcium and 800 IU of vitamin D3 daily for 3 years, the risk of hip fracture was reduced by 43%, and the risk of nonvertebral fracture by 32%. A 58% reduction in nonvertebral fractures was observed in 389 men and women over the age of 65 years who were receiving 700 IU of vitamin D3 and 500 mg of calcium per day (308).

A meta-analysis of seven randomized clinical trials that evaluated the risk of fracture in older persons given 400 IU of vitamin D3 per day revealed little benefit with respect to the risk of either nonvertebral or hip fractures (pooled relative risk of hip fracture, 1.15; 95% confidence interval [CI], 0.88 to 1.50; pooled relative risk of nonvertebral fracture, 1.03; 95% CI, 0.86 to 1.24). In studies using doses of 700 to 800 IU of vitamin D3 per day, the relative risk of hip fracture was reduced by 26% (pooled relative risk, 0.74; 95% CI, 0.61 to 0.88), and the relative risk of nonvertebral fracture by 23% (pooled relative risk, 0.77; 95% CI, 0.68 to 0.87) with vitamin D3 as compared with calcium or placebo (309). A Women's Health Initiative study that compared the effects of 400 IU of vitamin D3 plus 1000 mg of calcium per day with placebo in more than 36,000 postmenopausal women confirmed these results, reporting an increased risk of kidney stones but no benefit with respect to the risk of hip fracture.

The Women's Health Initiative study also showed that serum levels of 25-hydroxyvitamin D had little effect on the risk of fracture when levels were 26 ng per milliliter (65 nmol per liter) or less. However, women who were most consistent in taking calcium and vitamin D3 had a 29% reduction in hip fracture. Optimal prevention of both nonvertebral and hip fracture occurred only in trials providing 700 to 800 IU of vitamin D3 per day in patients whose baseline concentration of 25-hydroxyvitamin D was less than 17 ng per milliliter (42 nmol per liter) and whose mean concentration of 25-hydroxyvitamin D then rose to approximately 40 ng per milliliter (309).