THE CHARACTERIZATION OF APOPTOSIS IN SPONGIOTIC DISORDERS

Thesis
Submitted for the Partial Fulfillment of M.D. Degree of Dermatology and Andrology

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<td>Apaf-1</td>
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<td>Filaggrin</td>
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<td>Graft versus host disease</td>
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<td>Inhibitor of apoptosis protein</td>
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<td>Inflammatory dendritic epidermal cells</td>
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<td>126</td>
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<td>47</td>
<td>A case of dyshidrotic eczema showing weak Bcl2 expression (IHX40).</td>
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<td>48</td>
<td>A case of dyshidrotic eczema showing weak NF-κB expression (IHX40).</td>
<td>127</td>
</tr>
<tr>
<td>49</td>
<td>A case of nummular eczema showing weak p53 expression (IHX40).</td>
<td>127</td>
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INTRODUCTION

Programmed cell death plays a role in the homeostasis of the normal epidermis as well as in the terminal differentiation of keratinocytes resulting in a cornified layer formed by dead keratinocytes that is finally shed from the skin surface (Candi et al., 2005).

The regulation of apoptosis involves a dynamic interaction between death-accelerator and death-depressor proteins. Many molecules or genes involved in the regulation of apoptosis have been identified either by preventing or by promoting apoptosis. The ratio of antiapoptotic versus pro-apoptotic proteins determines the inherent susceptibility of a given cell to respond to apoptotic signals (Elmore, 2007).

The Fas, Bcl-2, nuclear factor kappa B (NF-κB) and p53 proteins are known to play a central role in the regulation of apoptosis. Fas is a cell surface receptor belonging to the nerve growth factor / tumor growth factor (TNF) receptor family, and is highly expressed on a variety of cells of lymphoid or nonlymphoid origin. FasL is a membrane protein, usually restricted to activated T cells and natural killer cells. Binding of FasL to Fas on Fas-sensitive target cells causes apoptosis of the target cells by triggering a caspase cascade (Choi et al., 2006).

p53 is a tumor-suppressor gene that controls cellular proliferation and can eliminate the cells by sending them down an irreversible apoptotic pathway (McNutt et al., 1994). On the other hand, NF-κB and Bcl-2 are protooncogenes that protect cells from apoptosis (Hockenberg et al., 1990).
Spongiosis refers to intercellular edema between the keratinocytes of the stratum malpighii. It is characterized by condensation of cells, widening of the intercellular space, and stretching of remaining intercellular contacts, resulting in a sponge-like appearance of the epidermis. Spongiotic dermatitis is a broad category of inflammatory skin disease in which spongiosis is the microscopic hallmark (Elder et al., 2005).

Although spongiosis refers only to serum between keratinocytes, the serous fluid is usually accompanied by exocytosis of inflammatory cells. In its common forms, including atopic dermatitis, allergic contact dermatitis and nummular dermatitis, spongiosis is usually accompanied by lymphocytes (lymphocytic spongiosis). In other forms, spongiosis may be accompanied by eosinophils or neutrophils (Machado-Pinto et al., 1996).

Apoptosis of keratinocytes has recently been implicated as a key mechanism of spongiosis which represents a main histopathologic feature in many dermatoses. In this process, caspases may play a role. Caspases are present in the cells as inactive zymogens that must be cleaved to generate free catalytic subunits able to associate and form active heterotetramers (Martinon & Tschopp, 2004).

Apoptosis of keratinocytes has been shown to be associated with cleavage of E-cadherin, an important component of adherence junctions. Therefore, cleavage of E-cadherin likely contributes to spongiosis formation. Because E-cadherin is a proteolytic target of caspase-3, it has been suggested that active caspase-3 cleaves E-cadherin in spongiotic disorders (Simon et al., 2006).
AIM OF THE WORK

The goal of our study was to evaluate the expression of apoptosis markers; caspase-3, Fas, Bcl-2, NF-κB and p53 in skin of patients with spongiotic disorders (atopic dermatitis, allergic contact dermatitis, irritant contact dermatitis, nummular eczema and dyshidrotic eczema) with respect to their role in the spongiosis formation.
APOPTOSIS

Background:

The term apoptosis (of Greek origin) means dropping off, as in leaves from a tree. The term apoptosis was first used by Kerr in 1972 to describe a morphologically distinct form of cell death (Kerr et al., 1972). Apoptosis has since been recognized and accepted as a distinctive and important mode of “programmed” cell death, which involves the genetically determined elimination of cells (Formigli et al., 2000). In 2002, Brenner, Horvitz and Sulston were awarded the Nobel Prize in Medicine for their discoveries concerning “genetic regulation of organ development and programmed cell death (Raj et al., 2006).

Apoptosis is a coordinated and often energy-dependent process that involves the activation of a group of cysteine proteases called “caspases” and a complex cascade of events that link the initiating stimuli to the final demise of the cell. The process is critical for organogenesis during embryonic development and for normal tissue homeostasis in the adult. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease (Noy, 2010).

Morphology of Apoptosis:

Apoptosis is characterized by specific features including cell membrane blebbing, cell shrinkage, chromatin condensation and DNA fragmentation (Figure 1). Pyknosis is the result of chromatin condensation and this is the most characteristic feature of apoptosis. The apoptotic cell appears as a round or oval mass with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments. Extensive plasma membrane blebbing occurs followed by karyorrhexis and
separation of cell fragments into apoptotic bodies during a process called “budding”. These bodies are subsequently phagocytosed by macrophages (Kurosaka et al., 2003).

Figure (1): Morphology of apoptosis (Kurosaka et al., 2003).

**Distinguishing Apoptosis from Necrosis:**

The alternative to apoptotic cell death is necrosis, which is considered to be a toxic process where the cell is a passive victim and follows an energy-independent mode of death. But since necrosis refers to the degradative processes that occur after cell death, it is considered by some to be an inappropriate term to describe a mechanism of cell death. Oncosis is therefore used to describe a process that leads to necrosis with karyolysis and cell swelling (Noy, 2010).

The type of stimuli and/or the degree of stimuli determines if cells die by apoptosis or necrosis. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia and cytotoxic anticancer drugs can induce
Review of Literature

apoptosis but the same stimuli can result in necrosis at higher doses. Although the mechanisms and morphologies of apoptosis and necrosis differ, there is overlap between these two processes. **Table 1** compares some of the major morphological features of apoptosis and necrosis (*Elmore, 2007*).

**Table (1): Comparison of morphological features of apoptosis and necrosis**

<table>
<thead>
<tr>
<th>Apoptosis</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single cells or small clusters of cells</td>
<td>Often contiguous cells</td>
</tr>
<tr>
<td>Cell shrinkage and convolution</td>
<td>Cell swelling</td>
</tr>
<tr>
<td>Pyknosis and karyorrhexis</td>
<td>Karyolysis, pyknosis, and karyorrhexis</td>
</tr>
<tr>
<td>Intact cell membrane</td>
<td>Disrupted cell membrane</td>
</tr>
<tr>
<td>Cytoplasm retained in apoptotic bodies</td>
<td>Cytoplasm released</td>
</tr>
<tr>
<td>No inflammation</td>
<td>Inflammation usually present</td>
</tr>
</tbody>
</table>

**Mechanisms of Apoptosis:**

The mechanisms of apoptosis are highly complex and sophisticated, involving an energy-dependent cascade of molecular events. Two general mechanisms are operative in apoptosis: one mechanism is triggered by the binding of death molecules to cell surface receptors (extrinsic or death receptor pathway), while the other is generated by signals arising within the cell (intrinsic or mitochondrial pathway) (*Hussein et al., 2004*).

However, there is evidence that the two pathways are linked and that molecules in one pathway can influence the other (*Igney & Krammer, 2002*). The extrinsic and intrinsic pathways converge on the same terminal, or execution pathway. This pathway is initiated by the
cleavage of caspase-3 and results in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, formation of apoptotic bodies and finally uptake by phagocytic cells (Martinval et al., 2005).

Caspases are cysteine proteases of the interleukin-1β-converting enzyme family, which are required for programmed cell death. Caspases are synthesized as zymogens with a prodomain followed by a large subunit and a small subunit. During the last decade, major progress has been made to understand caspase structure and function. The first known member of the caspase family was caspase-1, initially known as interleukin-1β-converting enzyme, an enzyme required for the maturation of IL1β (Cerretti et al., 1992). In subsequent years, a number of caspases have been cloned from various mammalian and non-mammalian species. Many of the cloned caspases, including caspase-1, do not have a role in apoptosis execution, whereas some caspases have dual functionality having roles both in apoptotic and nonapoptotic signaling (Kumar, 2007).

The structural studies predict that the mature caspase is a heterotetramer, composed of two heterodimers derived from two precursor molecules. In addition to the regions that give rise to the two subunits, procaspases contain N-terminal prodomains of varying lengths. Based on the length of the prodomain, caspases can be divided into two groups: those that have a relatively long prodomain and those containing a short prodomain. The long prodomains in many caspases consist of specific protein–protein interaction domains that play a crucial role in caspase activation (Lavrik et al., 2005).

These domains mediate recruitment of the procaspase molecules to specific death signaling complexes, leading to their autocatalytic
activation, by mechanism(s), the precise details of which are still a matter of some debate, but often termed ‘proximity-induced’ activation (Fuentes-Prior & Salvesen, 2004).

The large prodomains of procaspases contain structural motifs that belong to the so-called death domain superfamily. Death domains are 80- to 100-residue-long motifs involved in the transduction of the apoptotic signal. This superfamily consists of the death domain (DD), the death effector domain (DED), and the caspase recruitment domain (CARD) (Kumar, 2007).

Each of these motifs interacts with other proteins by homotypic interactions. All members of the death domain superfamily are characterized by similar structures that comprise 6 or 7 antiparallel amphipathic α-helices. Structural similarity suggests a common evolutionary origin for all recruitment domains. However, the nature of the homotypic interactions differs within the superfamily. DD and CARD contacts are based on electrostatic interactions, while DED contacts use hydrophobic interactions (Lavrik et al., 2005).

About half of the 14 known caspases play roles in apoptosis, and these can be classified in two groups. (a) Initiator or apical caspases, caspases 2, 8, 9, and 10, contain long prodomains that mediate interactions of the caspase with upstream adaptors and effectors (Elmore, 2007). All initiator caspases are characterized by the presence of a member of the DD superfamily (DED or CARD), which enables their recruitment into the initiation complex. Several activating complexes for initiator caspases have been reported so far. (b) Effector/executioner caspases, caspases 3, 6, and 7, contain short prodomains that are cleaved
by initiator caspases (Kumar, 2007). Executioner caspases catalyze downstream steps of apoptosis by cleaving multiple substrates including proteins that mediate and regulate apoptosis, proteins involved in DNA repair, and cell cycle–related proteins. The activation of the effector caspase cascade differs between extrinsic (death receptor–mediated) and intrinsic (mitochondria-mediated) pathways (Fuentes-Prior & Salvesen, 2004).

Caspase-9 is regarded as the canonical caspase in the intrinsic mitochondrial pathway and caspase-8 as the key initiator of death receptor-mediated apoptosis. The role of caspase-2 remains somewhat controversial and enigmatic. Caspase-3 is the most downstream enzyme in the apoptosis-inducing protease pathway, and is probably the most clearly associated with cell death (Abdel-Latif et al., 2008).

Cleavage of a procaspase at the specific Aspartate-X bonds results in the formation of the mature caspase, which comprises the heterotetramer p202–p102 and causes release of the prodomain. The overall architecture of all caspases is similar. Each heterodimer (p10–p20) is formed by hydrophobic interactions resulting in the formation of several parallel β-sheets, composed of 6 antiparallel β-strands. Two heterodimers interact via a 12-stranded β-sheet that is surrounded by α-helices (Fuentes-Prior & Salvesen, 2004).

The action of caspases is regulated on several levels, including blockade of activation of caspases at the death-inducing signaling complex (DISC) as well as inhibition of enzymatic caspase activity. c-FLIP proteins are well-known inhibitors of death receptor-induced apoptosis. There are 3 c-FLIP isoforms described on the protein level, c-
FLIPL, c-FLIPS, and c-FLIPR. Under conditions of overexpression, all isoforms inhibit activation of procaspase-8 at the DISC by blocking its processing. At the same time, there is increasing evidence that c-FLIPL, when present at the DISC at low concentrations, facilitates the cleavage of procaspase-8 at the DISC by forming c-FLIPL–procaspase-8 heterodimers (Golks et al., 2005).

In addition to their activation by proteolytic cleavage, the activity of caspases controlled at other levels including regulation of caspase expression levels, post-translational modifications, and direct association with inhibitory proteins. For example, the expression of caspase-6 and caspase-10 is transcriptionally regulated by the tumor suppressor p53 (Jin & El-Deiry, 2005).

The inhibitor of apoptosis (IAP) family of proteins includes 8 mammalian family members, including XIAP, c-IAP1, c-IAP2, and MLIAP/ livin. They specifically inhibit the initiator caspase-9 and the effector caspase-3 and -7. IAPs are not the only natural inhibitors of caspases. The baculoviral p35 protein is a pan-caspase inhibitor, and it targets most caspases, in contrast to IAPs, which affect only caspase-3, -7 and -9. The mechanism of caspase inhibition by p35 involves the formation of an inhibitory complex that is characterized by a protected thioester link between the caspase and p35 (Abdel-Latif et al., 2008).

Caspases, being the key effector molecules in apoptosis, are potential targets for pharmacological modulation of cell death. First, increased levels of caspase activity are often observed at sites of cellular damage in a number of diseases, including myocardial infarction, stroke, sepsis, and Parkinson. Inhibition of caspase activity for these diseases is
predicted to be therapeutically beneficial. Second, discovery of drugs that selectively inhibit inflammatory caspases (caspase-1, -4 and -5) may help to control autoimmune diseases like rheumatoid arthritis. Finally, selective activation of caspases would be an approach in the treatment of cancer and chronic viral infections (Lavrik et al., 2005).

A number of important issues and controversies remain unresolved in caspase biology. These include: (i) how to interpret and explain caspase redundancy and compensation, (ii) how some caspases can function in both apoptotic and nonapoptotic pathways and factors that regulate the alternative caspase function, (iii) what pathways of caspase activation exist in the absence of initiator caspases, (iv) whether physiological cell death in the complete absence of caspases is indeed programmed or entirely different from apoptosis, (v) what caspase targets are essential for dismantling apoptotic cells and (vi) how precisely are caspases activated and regulated in response to different apoptotic and nonapoptotic signals. The ongoing genetic studies using various model systems and careful in vitro biochemical and molecular analyses coupled with more reliable reagents may help to explore the unresolved issues and clarify some of the controversies in the caspase field (Kumar, 2007).

Extrinsic pathway:

The extrinsic signaling pathways that initiate apoptosis involve transmembrane receptor-mediated interactions. The binding of Fas ligand to Fas receptor results in the binding of the adapter protein Fas-associated death domain protein (FADD) and the binding of TNF ligand to TNF receptor results in the binding of the adapter protein TNF receptor associated death domain (TRADD) with recruitment of FADD and receptor-interacting protein-1 (RIP-1). FADD then associates with
procaspase-8 via dimerization of the death effector domain. At this point, a death-inducing signaling complex (DISC) is formed, resulting in the autocatalytic activation of procaspase-8. Once caspase-8 is activated, the execution phase of apoptosis is triggered (Diessenbacher et al., 2008).

In death receptor–mediated apoptosis, 2 types of signaling pathways have been established. Type I cells are characterized by high levels of DISC formation and increased amounts of active caspase-8. Active caspase-8 leads to the activation of downstream, effector caspase-3 and -7. In type II cells, there are lower levels of DISC formation and, thus, lower levels of active caspase-8. In this case, signaling requires an additional amplification loop that involves the cleavage of the Bcl-2–family protein Bid by caspase-8 (Korsmeyer et al., 2000).

**Intrinsic pathway:**

The intrinsic pathways involve a diverse array of non-receptor-mediated stimuli that produce intracellular signals that act directly on targets within the cell. The stimuli may act in either a positive or negative fashion. Negative signals involve the absence of certain growth factors, hormones and cytokines that can lead to failure of suppression of death programs, thereby triggering apoptosis. Other stimuli that act in a positive fashion include, but are not limited to, radiation, toxins, hypoxia, hyperthermia, viral infections, and free radicals (Elmore, 2007).

Intracellular death signals trigger the insertion of proapoptotic Bcl-2 proteins into the outer mitochondrial membrane, resulting in membrane permeabilization, release of proapoptotic mitochondrial factors such as cytochrome c, and impairment of mitochondria function.
Upon its cytoplasmic release, cytochrome c binds to apoptotic protease activating factor-1 (Apaf-1) to form an apoptosome, which recruits and activates procaspase 9 and initiates a caspase cascade (Danial & Korsmeyer, 2004).

The control and regulation of these apoptotic mitochondrial events occurs through members of the Bcl-2 family of proteins. The tumor suppressor protein p53 has a critical role in regulation of the Bcl-2 family of proteins. Mitochondrial damage in the Fas pathway of apoptosis is mediated by the caspase-8 cleavage of Bid. This is one example of the “cross-talk” between the death-receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway (Igney & Krammer, 2002).

**Phases of Apoptosis:**

According to recent understanding, the process of apoptosis can be subdivided into at least three different phases. During the initiation phase, apoptosis can be affected by a variety of extrinsic and intrinsic signals including, cytokines, hormones, growth factors, radiotherapy, UV, cytotoxic drugs and viruses. These triggers can activate inducers of apoptosis controlled by several regulators, the decision to die is then defined, and finally leads to the activation of effectors of apoptosis. It is assumed that the execution phase of apoptosis defines the decision to die at the point of no return of the apoptotic cascade. During the execution phase, the central executioner of apoptosis is activated (Noy, 2010).

Execution caspases activate cytoplasmic endonuclease, which degrades nuclear material, and proteases that degrade the nuclear and cytoskeletal proteins. Caspase-3, caspase-6, and caspase-7 function as effector or “executioner” caspases. Caspase-3 is considered to be the
most important of the executioner caspases and is activated by any of the
initiator caspases (caspase-8, caspase-9, or caspase-10). Caspase-3 will
cleave gelsolin (actin binding protein) and the cleaved fragments of
gelsolin, in turn, cleave actin filaments in a calcium independent manner.
This results in disruption of the cytoskeleton, intracellular transport, cell
division, and signal transduction (Kothakota et al., 1997).

**Molecular Regulation of Apoptosis:**

Many molecules or genes involved in the regulation or induction of
apoptosis have been identified (Figure 2):

1-Fas

Fas, also known as CD95 antigen, is a 48 kDa transmembrane
glycoprotein. It is a member of the nerve growth factor receptor/tumor
necrosis factor superfamily. This cell surface molecule mediates receptor-
triggered apoptosis that plays an important role in immune regulation,
development and progression of cancers (El-Bassiouni et al., 2006).

FasL, the ligand for Fas, is a 40-kDa glycosylated type 2
transmembrane protein that belongs to the TNF family which also
includes TNF-α, tumor necrosis factor-related apoptosis-inducing ligand
(TRAIL) and tumor necrosis factor-like weak inducer of apoptosis
(TWEAK) (Zimmermann et al., 2011). The death receptor ligands are
involved in many physiological and pathological processes involving
triggering of apoptosis and inflammation. These molecules are comprised
of a C-terminal extracellular portion, a transmembrane domain, and an N-
terminal domain (Weinlich et al., 2010).
Figure (2): Molecular regulation of apoptosis (Weinlich et al., 2010).

Binding of FasL to Fas at the cell surface causes the association of FADD and other proteins to the Fas cytoplasmic tail (death domain), via a homotypic death domain-death domain interaction. Caspase-8 is then recruited, which in turn initiates a caspase cascade resulting in the activation of caspase-3 and subsequent cleavage of proteins leading to the execution of apoptosis (Griffith et al., 1995).

Although FasL expression was initially confined to activated T cells, recent studies indicated that FasL is expressed widely in adult...
tissues, in particular neutrophils and activated lymphocytes, in immune privileged tissues and in certain tumor cells. Resting T cells do not constitutively express FasL, whereas activated T cells express FasL. Thus, when a Fas-expressing activated T cell comes in contact with another cell expressing FasL on its surface, it undergoes apoptosis. This Fas-mediated apoptosis provides a mechanism that eliminates cells that are no longer needed (Weinlich et al., 2010).

FasL plays an important role in the effector function of cytotoxic T lymphocytes and also regulates their homeostasis. Genetic mutations that inactivate either FasL or Fas are associated with autoimmune lymphoproliferative syndrome, a hereditary condition characterized by the accumulation of atypical lymphocytes and by the development of autoimmune manifestations (Straus et al., 1999). The FasL/Fas system has been found to exert functions different from apoptosis in certain cellular contexts. Engagement of Fas in immature dendritic cells triggered their maturation and expression of proinflammatory cytokines in the absence of apoptosis (Farley et al., 2006).

Epidermal keratinocytes express Fas, but not FasL. However, abnormal expression of active FasL was found in keratinocytes of patients with toxic epidermal necrolysis (TEN), suggesting that a suicidal keratinocyte reaction contributes to the pathogenesis of TEN (Viard et al., 1998). Squamous cell carcinomas were found to have decreased expression of Fas and increased expression of FasL, suggesting that this type of skin cancer may employ FasL/Fas system to evade immune surveillance and tumor lysis (Bachmann et al., 2001).
FasL was found to be involved in the pathogenesis of eczematous dermatitides. Vesicle formation in eczemas has been largely attributed to rupturing of keratinocyte attachments as a result of intercellular edema (Klunker et al., 2003). Recent findings suggest that keratinocyte death plays a major role in vesicle formation. This keratinocyte death appears to be apoptotic and to be mediated by FasL, delivered to the epidermis by infiltrating T lymphocytes and acting on Fas whose expression on the surface of keratinocytes is induced by T lymphocyte-derived IFN-γ. These findings clearly demonstrated the important role of FasL in the epidermal destruction in inflammatory skin diseases (Farley et al., 2006).

In addition to its role in the induction of apoptosis, Fas has been suggested to elicit antiapoptotic and proinflammatory signals in the epidermis through the production of epidermal growth factor (EGF) receptor ligands, such as amphiregulin, transforming growth factor-α (TGF-α) and numerous proinflammatory cytokines. Recent work shows that the intracellular domain of Fas can be tyrosine phosphorylated, which can lead to the recruitment and activation of phosphatidylinositol-3-kinase (PI-3K). These findings are based on in vitro studies of immortalized and primary human keratinocytes and other cell types; their relevance for the in vivo situation within the epidermis remained therefore unclear (Sancho-Martinez et al., 2009).

2-Bcl-2 family

Bcl-2 (the B-cell leukemia/lymphoma-2 gene) is a protein produced by a gene of the same name that resides at chromosome 18q21. This protein was initially discovered in patients with B-cell leukemia and follicular lymphoma who demonstrated increased synthesis of Bcl-2. Up to 90% of follicular lymphomas and some B-cell malignancies of
follicular center-cell lineage demonstrate increased production of this polypeptide. Bcl-2 is believed to confer a survival advantage on these neoplastic cells. The protein produced in hematopoietic malignancies is identical to that produced constitutively in normal tissues (Triscott et al., 1995).

The Bcl-2 is a proto-oncogene that was originally found as a result of its location at the site of a translocation between chromosomes 14 and 18. Its oncogenic potential has been attributed to its ability to inhibit apoptosis. Bcl-2 is the most prominent member of a group of proteins involved in the promotion and inhibition of cell death (Bcl-2 family). Although this polypeptide is classified as an oncogene, it exhibits no homology with other, more common oncogenes. Additionally, this protein is a member of a new category of oncogenes that prolong cell survival by inhibition of cell death rather than by encouraging cellular proliferation. This process may promote the accumulation of mutations in cells that would otherwise be eliminated (Hockenbery et al., 1990).

The Bcl-2 family of proteins is composed of antiapoptotic and proapoptotic proteins. Antiapoptotic Bcl-2 proteins (such as Bcl-2 or Bcl-xl) share four Bcl-2 homology or ‘BH’ domains (BH1–BH4). Proapoptotic Bcl-2 proteins can be separated into two subclasses: the Bax-like proteins (such as Bax and Bak), which contain three BH domains (BH1–BH3) and the BH3-only proteins (such as Puma or Noxa), which contain a single BH domain (BH3). Because many of these proteins are coexpressed in the same cells, the ratio of antiapoptotic versus pro-apoptotic proteins determines the inherent susceptibility of a given cell to respond to apoptotic signals (Elmore, 2007).
The Bcl-2 family of proteins controls the integrity of the outer-mitochondrial membrane (OMM). Some members, including Bcl-2 and Bcl-xL, maintain the integrity of the mitochondria to prevent the release of cytochrome c, whereas others, in particular Bax/Bak and the so-called BH3 only proteins, such as Bid and Bim, promote mitochondrial cytochrome c release. However, the mechanism by which the proapoptotic proteins of the Bcl-2 family induce the release of cytochrome c from mitochondria remains controversial. During apoptosis, permeabilization of the OMM leads to release of cytochrome c. Released cytochrome c binds to Apaf-1 triggering the formation of the apoptosome and the processing of caspase-9, thereby leading to apoptosis (Chipuk et al., 2010).

Although initially viewed as an oncogene, Bcl-2 has little mitogenic effect. Instead, its mitogenic potential has been attributed to its ability to inhibit apoptosis. Bcl-2 prolongs the survival of cells in the absence of survival factors by the blocking of apoptosis. In the skin, Bcl-2 is expressed in the basal keratinocytes but not in the suprabasal cells. It is thought that the main mechanism of action of the Bcl-2 family of proteins is the regulation of cytochrome c release from the mitochondria via alteration of mitochondrial membrane permeability (El-Domyati et al., 2007).

Considerable attention has been paid to the role that Bcl-2 plays in apoptosis. Though numerous reports have described the presence of this protein in cutaneous neoplasms, little attention has been paid to Bcl-2 in inflammatory disorders. Bcl-2 has been described in benign nevi, melanomas, blue nevi, Spitz nevi, basal cell carcinomas, Bowen’s disease, and Merkel cell carcinomas (Boyd et al., 1997). Bcl-2 expression
in inflammatory disease has been minimally evaluated. Nakagawa et al., 1994 studied biopsy samples of normal skin, psoriasis vulgaris, "chronic dermatitis," and Kaposi's sarcoma for the expression of Bcl-2. All were negative for Bcl-2.

Numerous inflammatory skin diseases demonstrate apoptotic keratinocytes. Bcl-2 expression in the skin will presumably suppress production of these cells. The pattern of expression of this protein in human skin has not been delineated nor are the promoters of its expression known. Wrone-Smith et al., 1995 found that keratinocyte cell cultures, normal skin, and psoriatic lesions were all negative for Bcl-2.

Conversely, the lack of epidermal Bcl-2 positivity of these tissues may be explained by the presence of inhibitors such as Bax and Bak. There is a 21% amino acid sequence homology between these two proteins and bax inhibits Bcl-2 activity through the formation of heterodimers. The pattern of staining by bax overlaps to some degree with that of Bcl-2, but in some tissues there are significant differences (Chipuk et al., 2010).

3-NF-κB

Nuclear factor kappa B (NF-κB) is one of the transcriptional factors that play a critically important role in regulation of cell cycle as well as influencing cell death pathways. There are five family members in mammals: RelA, c-Rel, RelB, NF-κB1 (p105/p50) and NF-κB2 (p100/p52). Both p105 and p100 are proteolytically processed by the proteasome to produce p50 and p52, respectively. Rel proteins bind p50 and p52 to form dimeric complexes that bind DNA and regulate
transcription. In unstimulated cells, NF-κB is sequestered in the cytoplasm by IκB inhibitory proteins (Whiteside et al., 1997).

The NF-κB/Rel transcription factors are present in the cytosol in an inactive state complexed with the inhibitory IκB proteins. Activation occurs via phosphorylation of IκBα at Ser32 and Ser36 followed by proteasome-mediated degradation that results in the release and nuclear translocation of active NF-κB. IκBα phosphorylation and resulting Rel-dependent transcription are activated by a highly diverse group of extracellular signals including inflammatory cytokines, growth factors and chemokines. Kinases that phosphorylate IκB at these activating sites have been identified (Karin & Ben-Neriah, 2000). NF-κB inducing kinase (NIK) and IκB kinase α (IKKα) regulate the phosphorylation and processing of NF-κB2 (p100) to produce p52, which is then translocated to the nucleus (Xiao, et al., 2001).

Phosphorylation culminates in the nuclear translocation of active Rel/NF-κB dimers, their binding to DNA and the transcriptional activation of cellular genes involved in immune responses, inflammation, viral infection, cell proliferation and survival. In turn, nuclear NF-κB factors trigger the resynthesis of IκBα, giving rise to an autoregulatory loop that terminates the activation process (Kato et al., 2003).

The Rel/NF-κB signaling pathway and the transcription factors that it activates have emerged as critical regulators of the apoptotic response. These proteins are best known for the key roles that they play in normal immune and inflammatory responses, but they are also implicated in the control of cell proliferation, differentiation, apoptosis and oncogenesis. In recent years, there has been remarkable progress in understanding the
pathways that activate the Rel/NF-κB factors and their role in the cell’s decision to either fight or surrender to apoptotic challenge (Diessenbacher et al., 2008).

Hundreds of NF-κB-regulated genes have been identified; the particular combination of NF-κB subunits within distinct NF-κB homo- or heterodimers is responsible for determining the subsets of genes that they activate. Experiments with cell lines deficient for NF-κB subunits revealed that in vivo specificity of cellular gene activation does not only lie within the sequence of the NF-κB DNA site, but is also likely to be greatly influenced by combinatorial protein–protein interactions with other promoter-bound factors. This additional level of regulation is likely to help determine the specificity of gene activation to ensure a differential response to different stimuli (Hoffmann et al., 2003).

Although a functional NF-κB-binding site was identified in the Bax promoter region, this site was dispensable for NF-κB-mediated suppression of Bax promoter activation by p53. Nevertheless, it is conceivable that suppression of Bax activation by NF-κB might contribute to the survival of some tumor cells, although the mechanism for this repression needs to be clarified. Finally, NF-κB and IκB subunits have been observed to localize to mitochondria, and to suppress mitochondrial gene expression (Cogswell et al., 2003).

The last few years have seen a virtual explosion in the number of studies investigating the role of NF-κB in apoptosis in different systems, and important progress was made in understanding the mechanisms involved. NF-κB activates the transcription of many genes capable of suppressing cell death (Burstein & Duckett, 2003).
In skin, NF-κB is proposed to protect epidermis against apoptosis by enhancing the expression of anti-apoptotic factors (*Abdou & Hanout, 2008*). Activation of NF-κB through Akt signaling may promote KCs survival by modulating expression of inhibitors of apoptosis protein (IAPs) or TNF receptor-associated factor (TRAF) death receptor adapters. Inhibition of NF-κB is required to sensitize cells to TNF-induced apoptosis (*Diessenbacher et al., 2008*).

There are several factors that influence the transcriptional activity and biological function of NF-κB and this in turn might affect its capacity to weigh in the balance between life and death. It is perhaps not surprising that in order to blunt the cell’s survival response, NF-κB is a substrate for caspase cleavage (*Barkett et al., 2001*).

### 4-P53

The p53 gene, located on the short arm of chromosome 17 acts as a tumor suppressor gene. P53 is a 393-amino-acid nuclear phosphoprotein that, in its natural form, can bind to DNA and prevent cells from entering the S (synthesis) phase of the cell cycle until repair of DNA damage or alternatively eliminate the cells by sending them down an irreversible apoptotic pathway (*El-Domyati et al., 2007*).

The tumor suppressor p53 has been implicated in a growing number of biological processes, including cell cycle arrest, senescence, apoptosis, autophagy, and aging and has a major impact on overall life span and stem cell reprogramming. Activation of p53 in response to oncogenic stress eliminates tumor cells by apoptosis or senescence. P53 is regulated at the protein level by post translational modifications such as phosphorylation and acetylation (*Farnebo et al., 2010*).
P53 can exert its apoptotic functions by inducing the expression of proteins involved in the mitochondrial apoptotic pathway (figure 3). In this regard, p53 can: (i) induce several proteins residing in the mitochondria, such as Bax, which belong to the Bcl-2 protein family and (ii) function to induce mitochondrial release of cytochrome c, which associates with Apaf-1 and caspase-9 to form the apoptosome. Moreover, Bax protein (a Bcl-2 protein homolog) heterodimerizes with Bcl-2, abrogates its function, and therefore promotes apoptosis (Hussein et al., 2003).

Figure 3: Schematic representation of the p53-dependent apoptotic pathways (Farnebo et al., 2010).
In skin, the primary keratinocyte (KC) signaling pathway leading to cell death is initiated by activation of mitogen activated protein (MAPK), followed by mitochondrial depolarization and caspase activation. This cell death pathway is promoted by p53 and Bax, which are minimally expressed in proliferating KCs. On the other hand, this pathway may be antagonized in normal KCs through expression of multiple antiapoptotic regulators including Bcl-x<sub>L</sub> (Bowen et al., 2004).

Considering the multiple roles of p53 in a wide range of biological processes and its ability to control life and death of cells, it is not surprising that p53 is tightly regulated. Posttranslational modifications such as phosphorylation and acetylation are clearly important for regulating p53 protein levels and activity. Nonetheless, recent studies have revealed critical regulatory circuits that target p53 at the RNA level via both RNA-binding proteins and regulatory RNAs. Proteins such as HuR, L26, RPL26, nucleolin, and Wig-1 can bind to the 5' or 3' untranslated regions (UTR) of p53 mRNA and control its stability or translation through various mechanisms. Wig-1 is induced by p53 and enhances p53 mRNA stability, thus forming a positive feedback loop. The microRNAs miR-125a and miR-125b downregulate p53 expression by recognizing response element in the 3'UTR of p53 mRNA (Zhang et al., 2009).

Mahmoudi et al., 2009 recently discovered a natural antisense transcript, Wrap53, that plays a key role in regulating the steady-state level of p53 mRNA by interacting with p53 5'UTR. The Wrap53 gene (encoding RNA antisense to p53) overlaps with the p53 gene on chromosome 17p13 in a head-to-head fashion. Wrap53α and p53 are co-expressed in cells. Interaction between the two transcripts via their
complementary regions protects p53 mRNA from degradation. Knockdown of Wrap53α or blocking Wrap53/p53 hybrid formation leads to reduced p53 mRNA levels. Conversely, overexpression of Wrap53α increases p53 mRNA levels and p53 protein expression. Thus, Wrap53α will potentiate p53-induced cell cycle arrest and/or apoptosis in response to cellular stress.

**Physiologic Apoptosis:**

The role of apoptosis in normal physiology is as significant as that of its counterpart, mitosis. It is estimated that to maintain homeostasis in the adult human body, around 10 billion cells are made each day just to balance those dying by apoptosis. Apoptosis is necessary to rid the body of pathogen-invaded cells and is a vital component of wound healing in that it is involved in the removal of inflammatory cells and the evolution of granulation tissue into scar tissue (Renehan et al., 2001).

Apoptosis is also needed to eliminate activated or auto-aggressive immune cells either during maturation in the central lymphoid organs (bone marrow and thymus) or in peripheral tissues. Oxidative stress plays a primary role in the pathophysiology of age-induced apoptosis via accumulated free-radical damage to mitochondrial DNA. It is clear that apoptosis has to be tightly regulated since too little or too much cell death may lead to pathology, including developmental defects, autoimmune diseases, neurodegeneration, or cancer (Elmore, 2007).

**Pathologic Apoptosis:**

Abnormalities in cell death regulation can be a significant component of diseases such as cancer, autoimmune diseases, ischemia,
and neurodegenerative diseases. Alterations of various cell signaling pathways can result in dysregulation of apoptosis and lead to cancer. The p53 tumor suppressor gene is a transcription factor that regulates the cell cycle and is the most widely mutated gene in human tumorigenesis (Wang & Harris, 1997).

**Assays for Apoptosis:**

Apoptosis assays, based on methodology, can be classified into six major groups:

1. **Cytomorphological alterations:**

   The evaluation of hematoxylin and eosin-stained tissue sections with light microscopy allows the visualization of apoptotic cells. Although a single apoptotic cell can be detected with this method, confirmation with other methods may be necessary. Because the morphological events of apoptosis are rapid and the fragments are quickly phagocytized, considerable apoptosis may occur in some tissues before it is histologically apparent. Additionally, this method detects the later events of apoptosis, so cells in the early phase of apoptosis will not be detected (Denda & Tsutsumi, 2011).

   Semi-ultrathin sections can be stained with toluidine blue or methylene blue to reveal apoptotic cells by standard light microscopy. This method depends on the nuclear and cytoplasmic condensation that occurs during apoptosis. Smaller apoptotic bodies will not be detected and healthy cells with large dense intracellular granules can be mistaken for apoptotic cells or debris. Additionally, there is loss of antigenicity during processing so that immunohistological or enzyme assays cannot be
performed on the same tissue. However, this tissue may be used for transmission electron microscopy (TEM) (*White & Cinti, 2004*).

TEM is considered the gold standard to confirm apoptosis. This is because categorization of an apoptotic cell is irrefutable if the cell contains certain ultrastructural morphological characteristics (*Elmore, 2007*). These characteristics are: (1) electron-dense nucleus; (2) nuclear fragmentation; (3) intact cell membrane even late in the cell disintegration phase; (4) disorganized cytoplasmic organelles; (5) large clear vacuoles; and (6) blebs at the cell surface.

2. DNA fragmentation:

The DNA laddering technique is used to visualize the endonuclease cleavage products of apoptosis. This assay involves extraction of DNA from a lysed cell homogenate followed by agarose gel electrophoresis. The terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) method is used to assay the endonuclease cleavage products. The DNA can then be labeled with a variety of probes to allow detection by light microscopy, fluorescence microscopy or flow cytometry. This assay is also very sensitive, allowing detection of a single cell via fluorescence microscopy or as few as 100 cells via flow cytometry (*Ito & Otsuki, 1998*).

3. Detection of caspases, cleaved substrates, regulators, and inhibitors:

There are more than 13 known caspases that can be detected using various types of caspase activity assays. There are also immunohistochemistry assays that can detect cleaved substrates. Caspase
activation can be detected in a variety of ways including western blot, immunoprecipitation and immunohistochemistry. Both polyclonal and monoclonal antibodies are available to both procaspases and active caspases (Talasz et al., 2002).

Apoptosis PCR microarray is a relatively new methodology that uses real-time PCR to profile the expression of genes involved in apoptosis. These PCR microarrays are designed to determine the expression profile of genes that encode key ligands, receptors, intracellular modulators, and transcription factors involved in the regulation of programmed cell death (Vallat et al., 2003).

4. Membrane alterations:

Externalization of phosphatidylserine residues on the outer plasma membrane of apoptotic cells allows detection via Annexin V with fluorescent microscopy. The advantages are sensitivity (can detect a single apoptotic cell) and the ability to confirm the activity of initiator caspases. The disadvantage is that the membranes of necrotic cells are labeled as well (Elmore, 2007).

5. Detection of apoptosis in whole mounts:

Apoptosis can also be visualized in whole mounts using dyes such as acridine orange, Nile blue sulfate, and neutral red. Since these dyes are acidophilic, they are concentrated in areas of high lysosomal and phagocytotic activity. The results would need to be validated with other apoptosis assays because these dyes cannot distinguish between lysosomes degrading apoptotic debris from degradation of other debris such as microorganisms (Zucker et al., 2000).
6. Mitochondrial assays:

Mitochondrial assays and cytochrome c release allow the detection of changes in the early phase of the intrinsic pathway. Laser scanning confocal microscopy creates submicron thin optical slices through living cells that can be used to monitor several mitochondrial events. Other mitochondrial dyes can be used that measure the redox potential or metabolic activity of the mitochondria in cells (Scorrano et al., 2002).

However, these dyes do not address the mechanism of cell death and should be used in conjunction with other apoptosis detection methods. Cytochrome c release from the mitochondria can also be assayed using fluorescence and electron microscopy. Apoptotic or anti-apoptotic regulator proteins such as Bax and Bcl-2 can also be detected using fluorescence and confocal microscopy (Zhang et al., 2002).

Apoptosis in Skin Biology:

Evidence is accumulating that apoptosis plays an important role not only in the pathogenesis of skin diseases, but is also involved in the homeostatic mechanisms in healthy skin. In this respect, terminal differentiation of keratinocytes is thought to be a special form of apoptosis, because there are similarities between terminally differentiating keratinocytes and apoptotic cells; for example, granular keratinocytes show signs of endonuclease activation and DNA fragmentation. Thus, it is likely that the proliferation of keratinocytes is regulated by apoptotic cell death to maintain a constant thickness of the epidermis (Teraki & Shiohara, 1999).

Apoptosis in the epidermis is a routine occurrence and is the source of various histopathologic findings. These include Civatte bodies, dark
Review of Literature

cells, sunburn cells, and colloid bodies. Apoptotic keratinocytes typically produce fewer budding apoptotic bodies, presumably because of their stiffer cell membrane structure (Raskin et al., 1997).

In no other organ system does apoptosis play so many vital roles as in the skin. Apoptotic cell death is critical for balancing of KCs proliferation as well as for formation of the stratum corneum. Apoptosis also represents an important cancer defense mechanism (Elmore, 2007).

Two notable cell survival pathways have been partially characterized in KCs. The first is a MAPK pathway triggered by EGF and phosphorylation of EGF receptor (EGFR). Signaling by EGF may also stimulate a second survival pathway involving phosphatidylinositol 3-kinase-mediated activation of the serine/threonine kinase Akt (protein kinase B). Apoptotic stimuli may activate multiple pathways simultaneously. Thus KCs must coordinate a delicate balance of many antiapoptotic and proapoptotic forces in responding to extracellular stimuli, shifting their equilibrium toward cell survival or cell death when one pathway predominates (Nickoloff et al., 2002).

At light microscopic level, apoptotic keratinocytes are characterized by a condensed & basophilic nucleus, and eosinophilic homogenization of the cytoplasm. Such individually dying cells are traditionally referred to by several histological terms, which include dyskeratotic cells, Civatte bodies, colloid bodies, satellite cell necrosis or sunburn cells. These cells represent distinctive subtypes of apoptotic keratinocytes (Nagata, 1997).
There are several mechanisms by which keratinocytes undergo apoptosis. First, activated cytotoxic T cells express FasL, which binds to Fas expressed on keratinocytes and results in apoptosis (Sayama et al., 1994). Apoptosis can also be induced via the release of effector cell granules, which include perforins and multiple serine proteases, called granzyme. Granzyme B can activate some of the caspase family members by proteolysis. Although Fas/FasL and perforin/granzyme can independently trigger the cell death program, the processes leading to apoptosis are similar in both pathways (Teraki & Shiohara, 1999).

**Dysfunctional apoptosis in skin disease:**

1. **Keratinocyte apoptosis and keratinocyte diseases:**

*Diseases of increased keratinocyte apoptosis:*

As sunburn cells represent the histologic manifestation of isolated KC apoptosis, clinical sunburn is the classic example of UVB-induced apoptosis. Although UVB activates both death receptor and mitochondrial apoptotic pathways in KCs, several skin diseases have been ascribed predominantly to excessive death receptor activation (Wehrli et al., 2000).

Apoptotic KCs are a feature of toxic epidermal necrolysis, cutaneous viral infections, alopecia areata, incontinentia pigmenti, lichen planus and other lichenoid reactions. Death receptor signaling is triggered in KCs by cytokines and direct contact with intraepidermal lymphocytes, via TNF and Fas receptors, respectively (Raj et al., 2006).

In acute eczematous dermatitis, KC apoptosis caused by dermis-infiltrating, activated T cells may play a crucial pathogenetic role in the development of spongiosis, the histopathological hallmark of acute
eczema. Remarkably, T-cell-mediated apoptosis of single KC, as well as spongiosis, is located predominantly in suprabasal epidermal layers (Armbruster et al., 2009).

*Diseases of decreased keratinocyte apoptosis:

Whereas diseases associated with increased KC apoptosis tend to be acute, those associated with decreased apoptosis tend to be chronic. In fact, most skin diseases or cutaneous lesions characterized by epidermal hyperplasia or hyperkeratosis likely involve decreased KC apoptosis. Psoriasis, verrucae, and skin cancer are the most notable examples (Bowen et al., 2004).

2. Melanocytes apoptosis and melanocytic tumors:

In normal skin, melanocytes constitutively express Bcl-2. Likewise, expression of Bcl-2 can be commonly observed in melanocytic nevi and malignant melanoma. However, no differences in Bcl-2 expression were found among various subtypes of benign and malignant melanocytic proliferation, suggesting that Bcl-2 cannot be considered as a marker of malignancy in melanocytic neoplasm (Jansen et al., 1998).

3. Hair cycle and hair loss:

Apoptosis is a central element in the regulation of hair follicle regression (catagen). In normal catagen, apoptotic cells are scattered in the outer root sheath and are engulfed quickly, initially not by macrophages but by nearby epithelial cells (Teraki & Shiohara, 1999).

4. Wound healing:

One of the interesting aspects of wound healing involves the transition from cell-rich granulation tissue to relatively cell-poor,
extracellular matrix rich scar tissue. Massive apoptosis of fibroblasts, endothelial cells, and pericytes is responsible for this transition. Aberrant apoptosis may play a role in pathologic wound healing (e.g., hypertrophic scars, keloids) (Raskin et al., 1997).

**Therapeutic and Clinical Applications of Apoptosis:**

Many antineoplastic agents exert their therapeutic effects via apoptosis. Unfortunately, these agents are rather nonspecific, and significant morbidity occurs because of apoptotic damage to healthy tissues. Paclitaxel has been shown to have some effect against malignant melanoma, inducing apoptosis in these and other neoplastic cells by interfering with mitosis (Wahl et al., 1996).

UV radiation has broad therapeutic applications. Investigations have demonstrated that PUVA selectively induces apoptosis in lymphocytes at doses that do not harm keratinocytes, offering an explanation for the clinical response that PUVA produces in lymphocyte-rich skin diseases such as CTCL and psoriasis (Vowels et al., 1996).

**Future Challenges and Directions of Apoptosis:**

Future challenges include unraveling the details of how apoptosis is controlled in epidermal development, and further understanding the factors that shift apoptotic balance in KCs in response to different stimuli. The clinical implications of this new knowledge will be development of new apoptosis-based therapeutics, directed toward protection of KCs from apoptosis in those diseases characterized by excessive KC cell death, or enhancement of KC apoptosis in diseases in which KC cell death is impaired (Raj et al., 2006).
Although inhibition of caspases and thus blocking cell death in the epidermis is a compelling idea, doubts about such a therapeutic approach are warranted. Inhibition of caspases will likely block apoptosis in the skin. This might result in repression of spongiosis \((\text{Farley et al., 2006})\). It cannot be excluded that caspase inhibition might result in increased inflammation because activated KCs are no longer eliminated from the skin as a source of proinflammatory cytokines \((\text{Kerstan et al., 2009})\).

Recently, a FasL-base pro-drug was described as a promising treatment against transformed cells. This pro-drug consists of an engineered inactive form of FasL which is activated by tumor-expressed metalloproteases. One of the major difficulties with this treatment is the proper delivery to tumor cells \((\text{Weinlich et al., 2010})\).

Furthermore, there are many approaches for discovery of mutant p53-targeting drugs. Forward chemical genetics relies on cellular screening to identify compounds with a desired phenotypic effect, such as induction of mutant p53-dependent apoptosis in tumor cells. Reverse chemical genetics, on the other hand, is based on screening to identify compounds that have direct effects on the target protein, for example increasing conformational stability of mutant p53. Molecular modeling can be used to identify compounds that potentially bind to specific sites on the target protein and affect its conformation and/or stability. The discovery of small molecules that target and reactivate mutant p53 proteins raises new hopes for more efficient cancer therapy with less severe side effects in the not so distant future \((\text{Farnebo et al., 2010})\).
SPONGIOSIS

Introduction:

Spongiosis refers to intercellular edema between the keratinocytes of the stratum malpighii, which gives a spongy appearance to the epidermis. Spongiotic dermatitis is a broad category of inflammatory skin disease in which spongiosis is the microscopic hallmark (Table 2). Although spongiosis refers only to serum between keratinocytes, the serous fluid is usually accompanied by exocytosis of inflammatory cells (Elder et al., 2005). In its common forms, including allergic contact dermatitis and nummular dermatitis, spongiosis is usually accompanied by lymphocytes (lymphocytic spongiosis) (Machado-Pinto et al., 1996).

The term eosinophilic spongiosis (ES) is used to describe foci of intercellular edema associated with intraepidermal eosinophils. ES was first used by Emmerson and Wilson-Jones to describe a distinctive epidermal reaction pattern seen in patients with early pemphigus whose biopsy specimens lacked obvious acantholysis (Emmerson & Wilson-Jones, 1968). Since then, ES has been associated with various other dermatoses, including bullous pemphigoid, allergic contact dermatitis, eosinophilic pustular dermatosis, erythema toxicum neonatorum, arthropod bite reaction and scabies (Hoss et al., 1996).

Spongiosis can also be accompanied by neutrophils. Neutrophilic spongiosis (NS) can be found in diseases such as dermatitis herpetiformis, acute generalized exanthematous pustulosis and intraepidermal blistering diseases. To date, a comprehensive review of the diseases that can display NS has not been performed (Leonard et al., 2008).
Table (2): Spongiotic disorders *(Elder et al., 2005)*

| 1. Spongiotic disorders with lymphocytes | • Atopic dermatitis  
• Allergic contact dermatitis  
• Irritant contact dermatitis  
• Nummular eczema  
• Dyshidrotic dermatitis  
• Seborreheic dermatitis  
• Polymorphous light eruption  
• Erythema multiforme  
• Erythroderma |
| 2. Spongiotic disorders with eosinophils | • Atopic dermatitis  
• Allergic contact dermatitis  
• Photoallergic drug eruption  
• Incontinentia pigmenti, vesicular stage  
• Erythema toxicum neonatorum |
| 3. Spongiotic disorders with neutrophils | • Seborreheic dermatitis  
• Dermatophytosis  
• Toxic shock syndrome |
| 4. Other disorders | • Vesiculobullous disorders e.g. fixed drug eruption, bullous pemphigoid, pemphigus, dermatitis herpetiformis  
• Pityriasis alba  
• TEN syndrome  
• Viral infection e.g. herpes simplex, varicella – zoster infection |
Spongiotic conditions that display eosinophilic and/or neutrophilic spongiosis:

Spongiotic dermatitides arise as vesicular, weepy papules, with spongiotic vesication histopathologically (acute spongiotic dermatitis); evolve into crusted, scaly papules and plaques, with spongiosis and serous crusting histopathologically (subacute spongiotic dermatitis); and eventuate as scaly, lichenified plaques, with little spongiosis and superimposed changes of lichen simplex chronicus (chronic spongiotic dermatitis) (Machado-Pinto et al., 1996).

The predominant inflammatory cells in spongiotic dermatitis are T-lymphocytes, although Langerhans' cells also proliferate and accumulate in spongiotic vesicles. Although lymphocytic spongiosis is prototypical of spongiotic dermatitis, ES and NS can also be encountered. It is commonly believed that eosinophils in the infiltrate of a spongiotic dermatitis indicate allergic contact dermatitis, but an infiltrate containing eosinophils and ES can be seen in other spongiotic dermatitides, including nummular dermatitis and spongiotic drug reactions, as well as other conditions in which spongiosis is conspicuous, such as dermatophytosis and scabietic dermatitis (Phelps et al., 2003).

The pattern of spongiotic dermatitis with eosinophils does raise the possibility of an immunobullous disorder in the differential diagnosis, and consideration can be given to performing a biopsy for direct immunofluorescence microscopy, depending on the clinical context. The presence of pronounced spongiosis in conjunction with intraepidermal and intracorneal neutrophils, including foci of NS, suggests the possibility of superficial fungal or bacterial infection in the differential diagnosis (Machado-Pinto et al., 1996).
There are five general categories of spongiotic dermatitis: 1) acute spongiotic dermatitis; 2) subacute-spongiotic dermatitis; 3) chronic spongiotic dermatitis; 4) lichen simplex chronicus; and 5) prurigo nodularis. The first three categories actually represent a pathologic continuum, whereas the last two are a pathologic response to trauma superimposed by the patient on the underlying dermatitis (Ackerman & Ragaz, 1984).

Eczematous disorders account for a large proportion of all skin disease, and constitute a major health problem worldwide. The clinical features include itching, redness, papules, vesicles, and scaling. Because T cells constitute a large population of cellular infiltrate in eczematous dermatitis, a dysregulated, cytokine mediated response of the immune system appears to be an important pathogenetic factor. Keratinocytes within eczematous lesions exhibit an unusual expression of MHC class II antigens, ICAM-1, and IFN-γ inducible protein-10. ICAM-1 mediates strong adhesion between T cells and KCs. Thus it seems probable that cytokines and cell surface molecules of the accumulated skin-infiltrating T cells affect KCs in situ (Trautmann et al., 2000 a).

In acute spongiotic dermatitis, the spongiosis is typified by massive intercellular edema of the epidermis with widening of the intercellular spaces. The edema produces disruption of desmosomal attachments, and as a consequence, numerous microvesicles form. In general, the degree of epidermal acanthosis is slight. Dermal edema is common, and it is often accompanied by a pronounced inflammatory infiltrate of mononuclear cells and eosinophils. A frequent accompaniment is a scale crust composed of neutrophils, plasma, and bacterial colonies i.e., so-called impetiginization (Phelps et al., 2003).
In subacute spongiotic dermatitis, the vesicles are smaller and there is greater acanthosis of the epidermis. Parakeratosis and hyperkeratosis are usually present, and the mixed dermal inflammatory infiltrate can still be prominent (Ackerman & Ragaz, 1984).

In chronic spongiotic dermatitis, the degree of spongiosis is mild and barely discernible, and the minute widening of the intercellular spaces between keratinocytes is only visible under higher magnification. There is a much greater degree of epidermal acanthosis, and there is often a thick, compact, hyperkeratotic horn with minimal parakeratosis (Machado-Pinto et al., 1996).

The major clinical entities that cause spongiotic dermatitis are: 1) atopic dermatitis; 2) allergic contact dermatitis; 3) irritant contact dermatitis; 4) nummular dermatitis; and 5) dyshidrotic dermatitis. The pathogenesis of spongiotic dermatitis in these entities is not fully appreciated, and it is likely that the various entities arise by different mechanisms. Perhaps the best described is allergic contact dermatitis (Weston & Bruckner, 2000).

**Atopic dermatitis**

Atopic dermatitis (AD) is a common complex disease that frequently follows a chronic relapsing course and affects the quality of life in a significant manner. It is often associated with elevated serum IgE levels and a personal or family history of type I allergies. AD occurs most commonly during early infancy and childhood and it is frequently associated with abnormalities in skin barrier function and allergen sensitization (Boguniewicz & Leung, 2010).
**Epidemiology:**

Atopic dermatitis (AD) is one of the most frequent chronic inflammatory skin diseases with an increasing prevalence, affecting 15% to 20% of children and 2% to 10% of adults in industrialized countries. About 45% of all cases of AD begin within the first 6 months of life, 60% begin during the first year, and 85% begin before 5 years of age. More than 50% of children who are affected in the first 2 years of life do not have any sign of IgE sensitization, but they become sensitized during the course of AD. As many as 70% of these children have a spontaneous remission before adolescence (*Rupec et al.*, 2010).

**Etiology and pathogenesis:**

The pathophysiology of AD remains incompletely understood, although gene-environment interactions in genetically predisposed individuals play a central role. AD may result from complex interactions between genetic susceptibility genes resulting in a defective skin barrier, defects in the immune system, and heightened immunologic responses to allergens and microbial antigens (*Zeppa et al.*, 2011).

- **Decreased skin barrier function:**

AD is associated with a marked decrease in skin barrier function due to the downregulation of cornified envelope genes (filaggrin and loricrin), reduced ceramide levels, increased levels of endogenous proteolytic enzymes, and enhanced trans-epidermal water loss. Mutations in filaggrin genes are strongly associated with increased AD risk, and probably account for around 10% of cases (*Williams & Grindlay*, 2009). Compared with patients without *FLG* mutations, those patients with AD who have mutations in *FLG* have disease that is earlier in onset, more
severe, and more persistent and more likely to be associated with asthma and allergic sensitization (*Boguniewicz & Leung, 2010*).

**Immunopathology of Atopic Dermatitis:**

Acute eczematous skin lesions are characterized by marked intercellular edema (spongiosis) of the epidermis. Dendritic antigen-presenting cells [e.g., Langerhans cells (LCs), macrophages] exhibit surface-bound IgE molecules. A sparse epidermal infiltrate consisting primarily of T lymphocytes is also frequently observed. In the dermis of the acute lesion, there is an influx of T cells with occasional monocyte-macrophages. The lymphocytic infiltrate consists predominantly of activated memory T cells bearing CD3, CD4, and CD45 RO. Eosinophils are rarely present in acute AD (*Leung et al., 2008*).

Chronic lichenified lesions are characterized by a hyperplastic epidermis with elongation of the rete ridges, prominent hyperkeratosis, and minimal spongiosis. There is an increased number of IgE-bearing LCs in the epidermis, and macrophages dominate the dermal mononuclear cell infiltrate. Mast cells are increased in number but are generally fully granulated. Increased numbers of eosinophils are observed in chronic AD skin lesions (*Bieber, 2010*).

**Cytokines and chemokines:**

Atopic skin inflammation is orchestrated by the local expression of pro-inflammatory cytokines and chemokines. Acute AD is associated with the production of T helper 2 type cytokines, notably IL-4 and IL-13, which mediate immunoglobulin isotype switching to IgE synthesis, and upregulate expression of adhesion molecules on endothelial cells. In
contrast, IL-5, is involved in eosinophil development and survival, and predominates in chronic AD (Toda et al., 2003).

*Key cell types in atopic dermatitis skin:*

1. **Antigen-presenting cells:**

   AD skin contains two types of high-affinity, IgE-receptor–bearing myeloid DCs: (1) LCs and (2) inflammatory dendritic epidermal cells (IDECs), IgE-bearing LCs that appear to play an important role in cutaneous allergen presentation to IL-4–producing Th2 cells (Novak et al., 2004). LCs that have captured allergen likely activate memory Th2 cells in atopic skin, but they may also migrate to the lymph nodes to stimulate naive T cells there to further expand the pool of systemic Th2 cells. Stimulation of FcεRI on the surface of LCs by allergens induces the release of chemotactic signals and recruitment of precursor cells of IDECs and T cells. Stimulation of FcεRI on IDECs leads to the release of high amounts of pro-inflammatory signals, which contribute to the amplification of allergic immune response (Niebuhr & Werfel, 2010).

2. **T cells:**

   AD is characterized by a biphasic reaction pattern. Whereas the initial phase is controlled by Th2 cytokines, the latter phase is dominated by Th1 cytokines. In acute AD, Th2 cells produce cytokines that enhance allergic skin inflammation. During the chronic phase of AD, Th1 cells produce IFN-γ that induces the activation and apoptosis of keratinocytes (Akdis et al., 2004).

   Recently, T regulatory (Treg) cells have been described as a further subtype of T cells that have immunosuppressive function and cytokine
profiles distinct from both Th1 and Th2 cells. Treg cells are able to inhibit the development of both Th1 and Th2 responses. The recent discovery that the IL-17-producing CD4^+ T cells (Th17 cells) can be found in the epidermis and dermal infiltrate of AD merits a role for this newly recognized T cell lineage in the pathogenesis (Kerstan et al., 2009).

3. Keratinocytes:

Keratinocytes play a critical role in the augmentation of atopic skin inflammation. AD keratinocytes secrete a unique profile of chemokines after exposure to pro-inflammatory cytokines. This includes high levels of RANTES after stimulation with TNF-α and IFN-γ. Keratinocytes also play a key role in the skin's innate immune responses via the expression of Toll-like receptors, production of pro-inflammatory cytokines and antimicrobial peptides (e.g. human β defensins and cathelicidins) in response to tissue injury or invading microbes (McGirt & Beck, 2006).

- Genetics:

AD is familially transmitted with a strong maternal influence. Although many genes are likely to be involved in the development of AD, there has been particular interest in the potential role of skin barrier/epidermal differentiation genes and immune response/host defense genes (Palmer et al., 2006). DNA microarray analyses have demonstrated downregulation of loricrin and filaggrin in AD (Barnes, 2010).

Clinical findings:

The clinical manifestations of AD vary with age; often three stages can be identified. In infancy, the first eczematous lesions usually emerge
on the cheeks and the scalp. Scratching, which usually starts a few weeks later, causes crusted erosions. During childhood, lesions involve flexures, the nape, and the dorsal aspects of the limbs. In adolescence and adulthood, itching may persist throughout the day and worsen at night, causing sleep loss and substantially impairing the patient's quality of life (Rupec et al., 2010).

The clinical phenotype of AD has been classified into the extrinsic and intrinsic types. The extrinsic and intrinsic types began to be adopted in the late 1980s. They are also called the allergic (classical) and non-allergic types. Since there is still no sufficient consensus whether the intrinsic type is a distinct entity, some researchers denominate it atopiform dermatitis (Tokura, 2010).

In 1980, Hanifin and Rajka introduced diagnostic criteria for the diagnosis of AD. In 1994, the United Kingdom Working Party developed more straightforward criteria for the diagnosis. More recently, clinical criteria for the diagnosis of AD were developed by the American Academy of Dermatology (Table 3) (Hon et al., 2008).

**The itch-scratch cycle:**

Pruritus is a major symptom of AD and affects the quality of life of patients in a significant manner. The clinical observation that pruritus in patients with AD is often not relieved by antihistamines suggests that mediators other than histamine, such as cytokines and neuropeptides, might be involved. IL-31, a cytokine that is increased in AD skin lesions, has been implicated in the development of chronic dermatitis through induction of severe pruritus (Boguniewicz & Leung, 2010).
Table (3): Universal Criteria for AD by American Academy of Dermatology.

<table>
<thead>
<tr>
<th>A. Essential features; must be present and, if complete, are sufficient for diagnosis:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pruritus</td>
</tr>
<tr>
<td>2. Eczematous changes that are acute, subacute, or chronic:</td>
</tr>
<tr>
<td>a. Typical and age-specific patterns</td>
</tr>
<tr>
<td>(i) Facial, neck, and extensor involvement in infants and children</td>
</tr>
<tr>
<td>(ii) Current or prior flexural lesions in adults/any age</td>
</tr>
<tr>
<td>(iii) Sparing of groin and axillary regions</td>
</tr>
<tr>
<td>b. Chronic or relapsing course</td>
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<table>
<thead>
<tr>
<th>B. Important features that are seen in most cases, adding support to the diagnosis:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Early age at onset</td>
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<tr>
<td>2. Atopy (IgE reactivity)</td>
</tr>
<tr>
<td>3. Xerosis</td>
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</tbody>
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<table>
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<tr>
<th>C. Associated features:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Keratosis pilaris/Ichthyosis/Palmar hyperlinearity</td>
</tr>
<tr>
<td>2. Atypical vascular responses</td>
</tr>
<tr>
<td>3. Perifollicular accentuation/Lichenification/Prurigo</td>
</tr>
<tr>
<td>4. Ocular/periorbital changes</td>
</tr>
<tr>
<td>5. Perioral/ periauricular lesions</td>
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</tbody>
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| D. Exclusions: Firm diagnosis of AD depends on excluding conditions such as scabies, allergic contact dermatitis, seborrheic dermatitis, cutaneous lymphoma, ichthyoses, psoriasis, and other primary disease entities. |

Pruritus may be intermittent throughout the day but is usually worse in the early evening and night. Its consequences are scratching, prurigo, lichenification and eczematous skin lesions. The itch-scratch cycle can lead to damage of the keratinocytes and release of intracellular
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antigens, which in a subset of patients could lead to a chronic autoreactive form of AD (Bieber, 2010).

Treatment:

- Topical therapy:

  1. Cutaneous hydration:

     Use of an effective emollient helps to restore and preserve the stratum corneum barrier, and may decrease the need for topical glucocorticoids. Moisturizers are available in the form of lotions, creams, or ointments. Some lotions and creams may be irritating due to added preservatives (Abramovits & Boguniewicz, 2006).

  2. Topical glucocorticoid therapy:

     Topical corticosteroids, first introduced in the early 1950s have been the mainstay of therapy for atopic dermatitis for many years (Hanifin et al., 2004). Because of potential side effects, most physicians use topical glucocorticoids only to control acute exacerbations of AD. However, recent studies suggest that once control of AD is achieved with a daily regimen of topical glucocorticoid, long-term control can be maintained in a subset of patients with twice weekly applications of topical fluticasone to areas that have healed but are prone to developing eczema (Haeck et al., 2011).

  3. Topical calcineurin inhibitors:

     Tacrolimus ointment is recognized as a drug that is highly suited for eruptions on the face and neck (Saeki et al., 2009). Tacrolimus ointment 0.03% has been approved for intermittent treatment of moderate to severe AD in children aged 2 years and older, with tacrolimus ointment
0.1% approved for use in adults; whereas pimecrolimus cream 1% is approved for treatment of patients aged 2 years and older with mild-moderate AD (Berger et al., 2006).

4. Other topicals:

Coal tar preparations may have antipruritic and anti-inflammatory effects on the skin. Newer coal tar products have been developed that are more acceptable with respect to odor and staining of clothes than some older products (Langeveld-Wildschut et al., 2000). There is limited evidence that the use of topical doxepin as short-term adjunctive therapy may provide slight relief of pruritus of limited duration. Sedation and contact allergies may complicate use, and therefore, it should be used for a limited duration (Hanifin et al., 2004).

- Identification and elimination of triggering factors:

  1. General considerations:

  Patients with AD are more susceptible to irritants than are unaffected individuals. Thus, it is important to identify and eliminate aggravating factors that trigger the itch-scratch cycle. These include soaps or detergents, contact with chemicals, smoke, abrasive clothing, and exposure to extremes of temperature and humidity (Leung et al., 2008).

  2. Specific allergens:

  Foods and aeroallergens such as dust mites, animal dander, molds, and pollens have been demonstrated to exacerbate AD. Potential allergens can be identified by taking a careful history and carrying out selective skin-prick tests or specific serum IgE levels (Greenhawt, 2010).
3. Emotional stressors:

AD patients often respond to frustration, embarrassment, or other stressful events with increased pruritus and scratching. Relaxation, behavioral modification, or biofeedback may be helpful in patients with habitual scratching (Arndt et al., 2008).

4. Infectious agents:

Anti-staphylococcal antibiotics are very helpful in the treatment of patients who are heavily colonized with S. aureus (Leung, 2003). Herpes simplex can provoke recurrent dermatitis and may be misdiagnosed as S. aureus infection. Antiviral treatment for cutaneous herpes simplex infections is of critical importance in the patient with widespread AD. Patients with dermatophyte infection may benefit from a trial of topical or systemic antifungal therapy (Boguniewicz & Leung, 2010).

- Pruritus:

Reduction of skin inflammation and dryness with topical glucocorticoids and skin hydration, respectively, often symptomatically reduce pruritus. Systemic antihistamines act primarily by blocking the H1 receptors, thereby ameliorating histamine-induced pruritus. However, histamine is only one of many mediators that can induce pruritus of the skin. Therefore, certain patients may derive minimal benefit from antihistaminic therapy. Some antihistamines are also mild anxiolytics and may offer symptomatic relief through sedative effects. Studies of newer, non-sedating antihistamines show variable results in the effectiveness of controlling pruritus in AD, although they may be useful in the subset of AD patients with concomitant urticaria or concurrent allergic rhinitis (Langner & Maibach, 2009).
**Review of Literature**

- **Phototherapy:**
  
  Broadband UVB, broadband UVA, narrowband UVB (311 nm), UVA-1 (340 to 400 nm), and combined UVA-B phototherapy can be useful adjuncts in the treatment of AD. The mechanism of action targets immunomodulation through apoptosis of inflammatory cells, inhibition of Langerhans cells and alteration of cytokine production (*Darsow et al.*, 2010). Photochemotherapy with psoralen and UVA light may be indicated in patients with severe, widespread AD, although studies comparing it with other modes of phototherapy are limited (*Gambichler*, 2009).

- **Systemic therapy:**
  
  1. **Systemic glucocorticoids:**

     The use of systemic glucocorticoids is rarely indicated in the treatment of chronic AD. The dramatic clinical improvement that may occur with systemic glucocorticoids is frequently associated with a severe rebound flare. Short courses of oral glucocorticoids may be appropriate for an acute exacerbation of AD whereas other treatment measures are being instituted (*Darsow et al.*, 2010).

  2. **Cyclosporine:**

     Cyclosporine is a potent immunosuppressive drug that acts primarily on T cells by suppressing cytokine transcription. The drug binds to cyclophilin and this complex inhibits calcineurin, a molecule required for initiation of cytokine gene transcription. Children and adults with severe AD, refractory to conventional treatment, can benefit from long-term low-dose cyclosporine treatment (*Haw et al.*, 2010).
3. Antimetabolites:

Mycophenolate mofetil is a purine biosynthesis inhibitor that has been used for treatment of refractory inflammatory skin disorders. Short-term oral mycophenolate mofetil results in clearing of skin lesions in adults with AD resistant to other treatment (Darsow et al., 2010). Methotrexate has been used for recalcitrant AD, although controlled trials are lacking. Dosing is more frequent than the weekly dosing used for psoriasis (Lyakhovitsky et al., 2010). Azathioprine is a purine analog with anti-inflammatory and antiproliferative effects; azathioprine has been used for severe AD, although no controlled trials have been reported (Darsow et al., 2010).

- Unproven therapies:
  1. Interferon-γ:

IFN-γ is known to suppress IgE responses and downregulate Th2 cell proliferation and function. It has been demonstrated that treatment with recombinant human IFN-γ results in clinical improvement. Reduction in clinical severity of AD correlated with the ability of IFN-γ to decrease total circulating eosinophil counts (Schneider et al., 1998).

  2. Biologics:

Biological agents present new therapeutic tools in the treatment of recalcitrant AD. They specifically target inflammatory cells and mediators, respectively, and thus may inhibit pathogenically relevant pathways. Approaches resulting in reduced T-cell activation using agents such as alefacept, rituximab and efalizumab have been shown to be effective in patients with moderate to severe AD (Krathen & Hsu, 2005).
Alefacept, which inhibits costimulation and induces apoptosis of T cells, leads to a significant improvement of symptoms. Interestingly, the depletion of B cells by rituximab resulted in a rapid and sustained reduction in skin inflammation, suggesting an important role of B cells in the pathogenesis of AD. Upon therapy with efalizumab, which inhibits T-cell recruitment, a significant decrease in symptoms has been reported. Anti-IgE (omalizumab) therapy showed beneficial effects in patients with moderate to severe AD; Anti-TNF-α (infliximab) has been shown to have short-term effects in patients with severe AD (Darsow et al., 2010).

3. Allergen immunotherapy:

Unlike allergic rhinitis and extrinsic asthma, immunotherapy with aeroallergens has not proven to be efficacious in the treatment of AD. A recent study of specific immunotherapy over 12 months in adults with AD sensitized to dust mite allergen showed improvement in SCORAD (Werfel et al., 2006).

Allergic contact dermatitis

Contact dermatitis is an inflammatory skin condition induced by exposure to an environmental agent. It is one of the most common occupational diseases. Two main types of contact dermatitis may be distinguished: (i) irritant contact dermatitis (ICD) and (ii) allergic contact dermatitis (ACD) (Nicolas et al., 2008). ACD results when a substance contacts skin that has undergone an acquired specific alteration in reactivity as a result of prior exposure of the skin to material eliciting dermatitis or to a chemically closely related substance (Hussein & Abdel-Magid, 2009).
Common causes of allergic contact dermatitis:

Nickel is the most common cause of ACD in women in almost all countries. The greater exposure of women to high-nickel content jewelry is a predisposing factor. Compulsory ingredient labeling of cosmetic products has greatly facilitated the diagnosis of cosmetic contact dermatitis (Goossens et al., 1999). Chromate is the most common contact allergen in men. Occupational exposure is most frequent in construction workers who handle cement (Saint-Mezard et al., 2004).

Contact dermatitis to clothes is usually located in the axillae, due to the release of allergens from the textile under the action of sweat and friction. A number of accelerators and antioxidants used in the production of synthetic rubber may also cause contact dermatitis (Saint-Mezard et al., 2004). Drug dermatitis may be elicited by the active ingredient of a topical drug, by the vehicle or by a preservative. Contact sensitization to antibiotics, and anesthetics is relatively frequent, especially in leg ulcers (Corazza et al., 2000)

Plant dermatitis can manifest itself in a variety of ways, depending upon the plant and the means of exposure. Urushiol, present in poison ivy is the most common cause of ACD in the United States, with 50% of the adult population clinically sensitive to it (Sasseville, 2009).

Pathophysiology of ACD:

1-Sensitization (afferent or induction) phase:

The sensitization phase occurs at the first contact of skin with a strong hapten. Through their proinflammatory properties, haptens activate innate immunity and deliver signals that induce the recruitment, migration and maturation of DCs. Haptens are processed within
cutaneous DCs and are expressed in the groove of MHC classes I and II molecules (Martin et al., 2008).

Hapten-bearing DCs migrate from the skin to the regional lymph nodes (LNs) where specific CD8+ and CD4+ T lymphocytes are primed. T cells proliferate and emigrate out of the LNs to the blood where they recirculate between the lymphoid organs and the skin. The sensitization step lasts 10–15 days (Akiba et al., 2002).

2-Elicitation (efferent or challenge) phase:

Re-exposure of sensitized individuals with the same hapten leads to the appearance of ACD within 24/72 h. Specific T lymphocytes are activated and trigger the inflammatory process. Recent studies have demonstrated that CD8+ cytotoxic T lymphocytes are the main $T_{\text{EFF}}$ of ACD, and that they are recruited early after challenge. Their activation induces the recruitment of leucocytes, which participate in the development of the eczematous lesions. The inflammatory reaction persists for few days and rapidly decreases following down-regulatory mechanisms (McFadden et al., 2011).

3-Regulation/resolution phase:

The resolution of ACD involves different mechanisms including the clearance of the hapten from the skin and the activation of CD4+ Tregs. However, the precise phenotype of Treg cells involved in the down-regulation of ACD as well as their site and mode of action still remain unknown (Vocanson et al., 2009).

Clinical hallmarks:

In acute phase, ACD is characterized by erythema and edema, followed by the appearance of papules, closely set vesicles, oozing and
crusting. In the chronic stages, involved skin becomes lichenified and fissured, but new episodes of oozing and crusting may occur. ACD is usually accompanied by intense pruritus (Krasteva et al., 1999).

**Histopathology:**

The histopathologic findings are different in acute and chronic ACD and are dependent on the severity of the inflammatory reaction. The most common histologic feature is spongiosis. It is often limited to the lower epidermis but, if the reaction is severe, it may affect the upper layers. The papillary vessels are dilated, with perivascular lymphohistiocytic infiltrate that may extend in the epidermis (exocytosis) and accumulates in the spongiotic vesicles. In subacute and chronic ACD the spongiotic pattern gradually fades out, the epidermis becomes hyperplastic, and parakeratosis develops (Saint-Mezard et al., 2004).

**Diagnosis:**

The site and clinical appearance of the lesions frequently suggest the etiologic factor. Dermatitis at the site of contact with jewelry, blue jeans buttons, wrist watches, and other metallic objects are seen in nickel dermatitis. A family history or a past history of atopy and psoriasis may be decisive particularly when a diagnosis of hand eczema is discussed (Van Der Valk et al., 2003).

Patch testing is the universally accepted method for the detection of the causative contact allergens. Patch testing is cost-effective if patients are selected on the basis of a clear-cut clinical suspicion of contact allergy and only if patients are tested with chemicals relevant to the problem. Patch tests are usually applied for 48 hours on the upper half of the back. Patches are read at least 20 minutes after their removal. It is
recommended to perform a second reading 24 or 48 hours after patch test removal (Suneja & Belsito, 2001). Descriptive Interpretation Scale of patch test recommended by the European and North American contact dermatitis groups is represented in Table 4 (Nelson & Mowad, 2010).

Table (4): Descriptive Interpretation Scale of patch test

<table>
<thead>
<tr>
<th>Grade</th>
<th>Meaning/appearance</th>
<th>Clinical relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Negative reaction</td>
<td>Excludes ACD. If ACD is still suspected, recheck technique or do ROAT.</td>
</tr>
<tr>
<td>R</td>
<td>Irritant reaction</td>
<td>Controls show similar response or there was an excited skin response.</td>
</tr>
<tr>
<td>?</td>
<td>Doubtful reaction</td>
<td>Negative test result. Repeat readings at 3, 4, and 7 days after patch removed.</td>
</tr>
<tr>
<td></td>
<td>Weak erythema only</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>Light erythema, nonvesicular</td>
<td>Equivocal test result. Could either be negative or indicative of waning prior sensitization. False-positive test result or excited skin syndrome must be ruled out by test in control subject.</td>
</tr>
<tr>
<td>++</td>
<td>Edema, erythema, discrete vesicles</td>
<td>Positive test result. Indicative of prior or current sensitization. Should correlate with history and physical findings. False-positive test result or excited skin syndrome must be ruled out by test in control subject.</td>
</tr>
<tr>
<td>+++</td>
<td>Coalescing vesiculobullous papules</td>
<td>Strongly positive result. Same conditions in grade ++ apply.</td>
</tr>
</tbody>
</table>
Treatment:

The only available etiologic treatment is elimination of the contact allergen. The patients should be informed about the identity of the offending agent and the possible sources of the sensitizer. Cross-reacting substances should be listed (Seyfarth et al., 2011). Topical steroids are used in the acute stage. Tacrolimus 0.1% ointment has demonstrated efficacy in the treatment of nickel-induced ACD. If ACD is widespread and severe, systemic corticosteroids may be indicated for a short period of time (Cohen & Heidary, 2004).

Phototherapy has been reserved for refractory patients who are unresponsive to corticosteroids and for patients who are unable to avoid all provoking factors in their daily environment (Cohen & Jacob, 2008).

Conventional immunosuppressive therapy is not appropriate in the management. Perspectives in pharmacological intervention include new classes of immunosuppressives, inhibitors of adhesion molecules, targeted skin application of regulatory cytokines and neutralization of pro-inflammatory cytokines (antisense oligonucleoïdes, anticytokine antibodies, soluble cytokine receptors) (Vocanson et al., 2009).

Irritant contact dermatitis

Irritant dermatitis is the most common form of dermatitis and is defined as nonspecific damage to the skin after exposure to an irritant. It is a non-immunologic response of the skin to direct chemical. Previously considered a monomorphous process, ICD is now understood to be a complex biologic syndrome, with diverse pathophysiology, natural history and clinical appearances (Thong & Maibach, 2008).
The incidence of ICD is difficult to determine because the accuracy of the epidemiologic data is limited. ICD accounts for 80% of all cases of contact dermatitis, and is often occupation-related. ICD caused by personal-care products and cosmetics is also common; however, very few of these patients with these irritant reactions seek medical help because they manage by avoiding the offending agent (Bordel-Gómez et al., 2010).

The clinical appearance of ICD varies depending on multiple external and internal factors. The spectrum is broad, ranging from mild redness and chapping to severe blistering and ulcerations. The classification of irritation is based on both morphology and clinical course of the dermatitis. Eleven phenotypes of ICD have been described (Table 5) (Chew & Maibach, 2005).

With sufficient concentration or duration of exposures, a wide range of chemicals is capable of acting as cutaneous irritants. Common cutaneous irritants include detergent, solvent and water. Not only the properties of the irritating substance (pH, solubility and detergent action) but also its physical state (gaseous, liquid or solid) is important. Important host factors include age, genetic background; the presence or absence of occlusion, sweating, pigmentation, dryness, sebaceous activity and the simultaneous presence of another skin disease or atopy. Important environmental factors include temperature, humidity, friction, pressure, occlusion and coexisting lacerations (Thong & Maibach, 2008).
### Table (5): Phenotypes of ICD

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Clinical manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acute ICD e.g. chemical burn</td>
<td>Acute onset, pruritus, burning &amp; pain, asymmetrical distribution, sharply demarcated</td>
</tr>
<tr>
<td>2. Delayed acute ICD e.g. Dithranol</td>
<td>Delayed onset (12–24 h or longer), burning sensation</td>
</tr>
<tr>
<td>3. Irritant reaction e.g. detergents</td>
<td>Acute onset, often multiple exposure, erythematous, monomorphic appearance</td>
</tr>
<tr>
<td>4. Chronic ICD e.g. hand eczema</td>
<td>Slowly developing, persist &gt;6 weeks, erythema, xerosis, fissure, ill-defined, pruritus and pain</td>
</tr>
<tr>
<td>5. Traumatic ICD e.g. manual worker</td>
<td>Slowly developing after preceding skin trauma, erythema, vesicles, papules and scaling</td>
</tr>
<tr>
<td>6. Acneiform ICD e.g. chloracne</td>
<td>slowly developing (weeks to months), pustules</td>
</tr>
<tr>
<td>7. Non-erythematous irritation e.g. mild irritants</td>
<td>Slowly developing, subtle skin damage not clinically visible</td>
</tr>
<tr>
<td>8. Subjective irritation e.g. lactic acid</td>
<td>Acute onset, itching, burning, tingling sensations but lack of objective signs on clinical examination</td>
</tr>
<tr>
<td>9. Friction dermatitis e.g. paper work</td>
<td>Slowly developing, erythema, scaling, fissuring and itching surrounding area of frictional contact</td>
</tr>
<tr>
<td>10. Asteatotic irritant eczema e.g. wet-workers</td>
<td>Slowly developing, xerosis, ichthyosiform scale, with eczematous changes, tightness and pruritus</td>
</tr>
<tr>
<td>11. Photoirritation e.g. psoralen</td>
<td>Acute onset, exposure to phototoxic agent</td>
</tr>
</tbody>
</table>
No diagnostic test exists for ICD. The diagnosis lies on the exclusion of other cutaneous diseases (especially ACD) and on the clinical appearance of dermatitis at a site exposed to cutaneous irritant. **Rietschel** has proposed criteria with subjective and objective features for the diagnosis of ICD. The more features identified, the stronger the case for ICD (Table 6) (*Rietschel, 2004*).

**Table (6): Diagnostic criteria of ICD**

<table>
<thead>
<tr>
<th>Major</th>
<th>Minor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subjective</strong></td>
<td></td>
</tr>
<tr>
<td>Onset within minutes to hours</td>
<td>Onset within 2 weeks of exposure</td>
</tr>
<tr>
<td>Pain, burning, stinging, or discomfort exceeding itching</td>
<td>Many people in the environment affected similarly</td>
</tr>
<tr>
<td><strong>Objective</strong></td>
<td></td>
</tr>
<tr>
<td>Macular erythema, hyperkeratosis, or fissuring</td>
<td>Sharp circumspection of the dermatitis</td>
</tr>
<tr>
<td>Glazed, parched, or scalded appearance of the epidermis</td>
<td>Evidence of gravitational influence, such as dripping effect</td>
</tr>
<tr>
<td>Healing process begins promptly on withdrawal of the offending agent</td>
<td>Lack of tendency of the dermatitis to spread</td>
</tr>
<tr>
<td>Patch testing is negative</td>
<td>Morphologic changes suggesting small concentration differences or contact time produce large differences in skin damage</td>
</tr>
</tbody>
</table>

Irritants cause disruption of skin barrier, leading to disruption of the lipid bilayers, with loss of cohesion of corneocytes, disturbance of lamellar body lipid extrusion, desquamation, and increased
 transepidermal water loss (TEWL). Barrier disruption often leads to structural changes in keratinocytes and cytokine release which includes IL-1α, TNF-α, IL-6, IL-8, IL-2 and IFN-γ, followed by inflammatory cell infiltration, increased epidermal turnover and cytotoxicity and apoptosis of keratinocytes (Nosbaum et al., 2009).

Although the later stages can appear histologically identical to ACD, the earlier stages are often different. Early, there may be intracellular edema of keratinocytes or there may be scattered necrotic keratinocytes throughout the epidermis. Neutrophils may be seen either in the epidermis or dermis, admixed with mononuclear cells and eosinophils. In acute ICD, The most commonly observed changes are intracellular vacuolation and nuclear pyknosis. Dermal changes include disruption and/or degeneration of collagen, Edema, mast cells degranulation and dilatation of blood vessels and lymphatics (Willis, 2002).

Unlike acute ICD, chronic ICD displays a relatively monomorphic histopathology, with changes including hyperkeratosis, parakeratosis, acanthosis and focal areas of mild spongiosis, exocytosis and minimal inflammatory infiltration primarily of mononuclear cells (Thong & Maibach, 2008).

Patch testing is often essential to help distinguish ACD from ICD or to diagnose concomitant ICD and ACD. Negative patch tests may favor a diagnosis of ICD "by exclusion" of ACD. A diagnosis of ACD may be missed with false-negative patch test results. Conversely, patch testing with obvious irritants, or non-standard chemicals or mixtures can lead to false-positive patch test results. Irritant patch test reactions may
present as erythema with or without papules and often remain confined to the test site and are sharply demarcated (*Rietschel, 2004*).

ICD is a risk factor for the development of ACD because the impaired skin barrier may increase the potential for the induction and elicitation of ACD. Thus, the prevention of irritant dermatitis means simultaneous prevention of ACD. Once an irritant has been identified as the causal factor, patients should be educated about irritant avoidance, including everyday practices that may cause or contribute to the ICD. Moisturizers increase hydration or prevent TEWL, thereby maintaining skin barrier function and reducing the risk of ICD (*Loffler & Effendy, 2002*).

Identification and elimination of the irritants and protection from further exposure are important in the management of ICD. The role of topical corticosteroids is controversial, but they may be helpful because of their anti-inflammatory effect. Emollients may improve barrier repair in dry, lichenified skin. Topical calcineurin inhibitors may be used as an alternative to topical corticosteroids in chronic ICD (*Belsito et al., 2004*).

In severe or chronic cases, phototherapy (PUVA or UVB) or systemic drugs, such as azathioprine and cyclosporine, may be effective. Grenz radiotherapy is a potential third-line adjunctive treatment. Bacterial superinfection can be treated with topical or systemic antibiotics (*Wilkinson & Beck, 2004*).
Nummular dermatitis

Nummular dermatitis, also known as discoid eczema, is a common type of endogenous eczema. It was first described by Rayer in 1845 but the term was introduced by Devergie in 1857. Nummular dermatitis is predominantly a disease of adulthood. Men are more frequently affected than women. The peak incidence in both males and females is around 50 to 65 years of age. There is a second peak in women around 15 to 25 years of age. Nummular eczema is rare in infancy and childhood. The peak age of onset in childhood is 5 years (Burgin, 2008).

It is characterized by multiple well-demarcated coin-shaped exudative papulovesicular lesions and plaques. Pinpoint oozing and crusting eventuate and are distinctive. Crust may however cover the entire surface. Plaques range from 1 to 3 cm in size. The surrounding skin is generally normal, but may be xerotic. Pruritus varies from minimal to severe. Central resolution may occur, leading to annular forms. Chronic plaques are dry, scaly, and lichenified (Wallengren, 2004).

The classic distribution of lesions is the extensor aspects of the extremities. In women, the upper extremities, including the dorsal aspects of the hands, are more frequently affected than the lower extremities. Exudative discoid and lichenoid dermatitis of Sulzberger-Garbe may represent a variant of nummular eczema. The course of the disease is usually continuous and relapse may frequently occur after occasional remissions (Burgin, 2008).

The pathogenesis of discoid eczema is not known. Some authors have found a high incidence of atopic background in their patients, but others have not, and the levels of IgE are usually within the normal range.
Unlike atopic eczema, there does not appear to be a clear association with atopy. Although xerosis has been reported in patients with discoid eczema, dry skin is not present in all patients (Krol & Krafchik, 2006).

Discoid eczema has been reported in association with occult infections, environmental allergens and various medications. The role of infection previously received much attention in the literature. An internal focus of infection, including teeth, upper respiratory tract, and lower respiratory tract, was found in 68% of patients in one study (Wallengren, 2004). A role for environmental allergens, such as the house dust mite and Candida albicans has also been touted. Generalized nummular eczema is seen in patients with hepatitis C undergoing combination therapy with interferon-α2b and ribavirin (Shen et al., 2005).

Patients are at increased risk of developing secondary contact dermatitis due to impaired barrier function that contributes to the chronicity and severity of dermatitis. Therefore, it is recommended that patients with chronic, recalcitrant nummular dermatitis be patch tested (Shankar & Shrestha, 2005).

Histopathologic changes are reflective of the stage at which the biopsy is performed. Acutely, there is spongiosis, with or without spongiotic microvesicles. In subacute plaques, there is parakeratosis, scale-crust, epidermal hyperplasia, and spongiosis of the epidermis. There is a mixed cell infiltrate in the dermis. Chronic lesions may resemble lichen simplex chronicus microscopically (Burgin, 2008).

First-line therapies such as emollients, topical corticosteroids and wet dressings are effective in the majority of cases. Mid- to high-potency topical corticosteroids and emollients are considered the mainstay of...
treatment in discoid eczema. Other topical agents such as calcineurin inhibitors and tar preparations have also been reported (Cox, 2006).

For more widespread involvement narrow-band UVB may be effective. More severe disease may not respond to these topical measures and requires systemic therapy. Unfortunately, there are limited data on the use of systemic therapy in discoid eczema. Oral antihistamines are useful if pruritus is severe. Oral antibiotics are indicated when secondary infection is present. Cyclosporine, azathioprine and mycophenolate mofetil have been proposed as potential therapy. Methotrexate should be considered in moderate to severe discoid eczema that has failed to respond to conventional therapies (Roberts & Orchard, 2010).

**Dyshidrotic dermatitis**

The term of “dyshidrosis” was given by Fox in 1873 as being related to “difficult sweating” and characterized by recurrent crops of vesicles or bullae located on the lateral aspects of the fingers and on the palms and soles (Fox, 1873). Hutchinson, describing the same entity in 1876, added the term cheiro-pompholyx, or vesicular eruption of the hands, but discounted sweating (Hutchinson, 1876). Ever since, the terms dyshidrosis and pompholyx have been used to describe the same condition, different conditions, or simply any chronic and often disabling vesicular hand eczema (despite the fact that episodic recurrences and absence of erythema are included in the classic definitions of both dyshidrosis and pompholyx) (Storrs, 2007).

Dyshidrosis and pompholyx are distinct but rare entities. The former is characterized by the sudden development of small (1-2-mm)
vesicles on nonerythematous skin, usually on the sides of the fingers or on the palms and soles. The vesicles itch a bit, last 1 to 2 weeks, desquamate, and then recur at unpredictable intervals. Pompholyx is similar but more severe, explosive, includes bullae, and usually involves the palms but rarely the soles (Guillet et al., 2007).

Clearly, the terms pompholyx and dyshidrosis are obsolete in that really no modern investigators use them as originally defined. Some dermatologists define them rigidly for use in outpatient clinics, but in studies and reviews such rigidity seldom prevails. Until a more concise label can be agreed on, the use of “acute and recurrent vesicular hand dermatitis,” given by Veien & Menne, 2000, is proposed for discussion (Storrs, 2007).

Pompholyx can develop on the basis of atopy, contact irritation and contact sensitization, id reaction and as a side effect of drug treatment. Contact allergy is common in patients affected with vesicular palmoplantar eczema but the causal relationship is not always clear. There are cases in which contact allergy has exacerbated pre-existing dermatitis. However, in other cases, the causal relationship may be the reverse. The impaired barrier function in vesiculobullous hand dermatitis may in some cases lead to sensitization and a higher prevalence of contact dermatitis in the affected population (Lehucher-Michel et al., 2000).

In acute pompholyx, there is an explosive outbreak of deep-seated vesicles on the palms, the lateral aspects of the fingers, and the soles, usually in a symmetric pattern. Discomfort and itching usually precede the development of the blisters. Blisters may coalesce then desiccate and resolve without rupture. Intact, large blisters can be drained, but should
not be unroofed. However, large blisters may rupture spontaneously, leaving oozing or dried up erosions. This acute phase is followed by desquamation of the affected areas. Individual outbreaks are usually self-limited over 2 to 3 weeks, although they may recur. Attacks are most common among adolescents and young adults and seem to be more common in the spring and summer months (*Daven & Alexa, 2008*).

Despite its name, there is no histologic evidence that the acrosyringia of sweat glands are involved in the development of dyshidrosis. The histology depends on the chronicity of the disease. The primary vesicle appears as an intraepidermal spongiotic vesicle. Lymphocytic infiltration is common in the epidermis, with a mixed infiltrate observed in the dermis. In more chronic cases, the epidermis may show hyperproliferation, hyperkeratosis, or even psoriasiform epidermal hyperplasia (*Guillet et al., 2007*).

Established treatments, both topical and systemic, are limited in efficacy, risk: benefit ratio and prevention of further relapses. The cornerstones of topical therapy are corticosteroids, although calcineurin inhibitors also seem to be effective. Bexaroten gel has efficacy in chronic hand dermatitis including pompholyx. Another evolving treatment is the intradermal injection of botulinum toxin. The major disadvantage of botulinum toxin (Botox) is the need for injections, but efforts are being made to develop a topical form of application (*Wollina, 2010*).

Systemic therapy is often necessary in bullous pompholyx and in advanced cases of pompholyx. Corticosteroids are commonly used although no controlled study has been published to date. For recalcitrant cases, corticosteroids are combined with immunosuppressants. New types
of anti-inflammatory oral drugs such as leukotriene inhibitors and phosphodiesterase-4 (PDE4) inhibitors have become available. These seem to have potential in the adjuvant treatment of pompholyx. Monoclonal antibodies of various types have been investigated in small series, but have failed to demonstrate consistent efficacy (Wollina, 2009).

Topical photochemotherapy with methoxsalen is as effective as systemic photochemotherapy or high-dose UVA-1. Recently, UV-free phototherapy has been introduced, but more data are needed before final conclusions can be drawn. Radiotherapy might be an option for selected patients not responding to conventional treatment. In practice, patients benefit most from a combination of treatments (Arias-Santiago et al., 2010).
APOPTOSIS AND SPONGIOSIS

Recent findings have implicated apoptosis in mediating the death of keratinocytes in spongiotic lesions. Few studies were conducted to understand the role of apoptosis in pathogenesis of spongiotic disorders. However most of these studies focus on the relation between the apoptosis and pathogenesis of these disorders and not on spongiosis itself. Moreover, most of these studies were conducted on one or few disorders using one or two of the apoptotic regulatory molecules.

The consensus among many dermatopathologists and a few dermatologists is that the expression eczema should be replaced with the term “spongiotic dermatitis.” Spongiosis refers to the histopathologic changes that underlie most of the so-called eczemas, i.e., edema between the keratinocytes of the stratum malpighii, which gives a spongy appearance to the epidermis. Although most authors have accepted this concept, it is actually a gross oversimplification. Spongiosis occurs in numerous other dermatoses that are not, by conventional wisdom, classified as “eczema.” Examples include, but are not limited to pityriasis rosea, Gianotti Crosti syndrome, the annular erythemas, miliaria, Grover’s disease, polymorphous light eruptions, popular urticaria, lichen striatus, pemphigus and some pigmented purpuras (Phelps et al., 2003).

Spongiosis is characterized by condensation of cells, widening of the intercellular space, and stretching of remaining intercellular contacts, resulting in a spongelike appearance of the tissue. Specific adhesiveness of keratinocytes is provided by homophilic interactions of the cadherin superfamily (Koch et al., 1999).
Adherens junctions anchor actin microfilaments and contain E-cadherin (E-cad) as their transmembrane glycoprotein. The intracellular segment of E-cad associates with α-catenin, β-catenin, and γ-catenin (plakoglobin). The major transmembrane components of desmosomes are desmogleins (Dsg) and desmocollins (Dsc). These desmosomal cadherins bind to the cytoplasmic proteins plakoglobin and desmoplakin, and are linked to keratin intermediate filaments (Amagai, 1995). Although central to cellular adhesion, cadherins display physiologic functions beyond the mechanical interconnection of cells. Recent studies suggested that cadherins play a crucial role in regulatory pathways involved in various aspects of cell fate including developmental decisions, cell differentiation, and cell survival (Hugo et al., 2011).

The development of spongiosis is initiated by early keratinocyte apoptosis due to cell shrinkage and cleavage of E-cad, which is essential in mediating keratinocyte cohesion. Impairment and loss of keratinocyte cohesion constitute the primary event in spongiosis formation. Therefore, despite being the obvious driving force of spongiosis formation, fluid influx into the skin is apparently not the primary step, but rather the end result of a sequence of pathogenic events (Hugo et al., 2011). Accordingly, dermal inflammation and intense fluid influx into the dermis in urticaria leave skin coherence totally intact (Natbony et al., 1983).

In contrast, in early lesions of bullous autoimmune skin diseases in which desmosomal cadherins are targeted by autoantibodies, spongiosis is visible. It should be noted that spongiosis is a nonspecific sign of cutaneous inflammation involving the epidermis. It is found in all kinds
of eczemas, in bullous skin diseases, and in some viral and superficial fungal infections as well (Hertl, 2000).

Spongiosis takes place mainly in the spinous layer of the epidermis. The heterogenous basal layer contains stem cells, transit amplifying cells, and postmitotic differentiating cells with high expression of integrins. It seems that in the basal layer at least stem cells exhibit strong antiapoptotic defenses (Kaur & Li, 2000). In contrast to adherens junctions that may contain only E-cad, desmosomes always include cadherins from two subfamilies, Dsg and Dsc (Chitaev & Troyanovsky, 1997).

Apoptosis-induced protein cleavage in keratinocytes is selective for certain adherens junction and desmosomal proteins. E-cad is cleaved, whereas β-catenin and desmosomal cadherins are not. The functional properties of E-cad and desmosomal cadherins are distinct despite their overall structural homology. The most striking sequence difference between Dsc, Dsg and E-cad lies in their cytoplasmic tails (Hanakawa et al., 2000). This may contribute to the selectivity of the cytoplasmic tails for different plaque proteins connecting them with different cytoskeletal filaments. These differences may also account for the differential behavior of desmosomal cadherins and E-cad in keratinocyte apoptosis (Hugo et al., 2011).

In the spongiotic epidermis of eczematous dermatitis, not all keratinocytes go into apoptosis. Therefore it is likely that in areas of intense spongiosis there is additional cleavage of cadherins on bystanding keratinocytes without ongoing apoptosis possibly due to proteinases released from secondary necrotic keratinocytes. The β-catenin binding
domain of E-cad has been mapped to the residues 815±819 in the cytoplasmic tail (*Miller & Moon, 1996*).

Recently, it was demonstrated that truncation of E-cad, loss of β-catenin binding, and cellular dissociation take place during prostate and mammary involution (*Mohamet et al., 2011*). In the same study E-cad mRNA was examined by northern blot and reverse transcriptase polymerase chain reaction and only the accumulation of a single transcript was found. Together these results demonstrate that loss of E-cad results from rapid, posttranslational cleavage of the molecule, and not from transcriptional events.

The early apoptotic response of keratinocytes is characterized by cleavage of E-cad, whereas desmosomal cadherins remain intact. Hydrostatic pressure, which is an important factor in the development of spongiosis, and the portions of the epidermal cell surface that still retain desmosomes may explain the elongation and distortion of remaining intercellular contacts observed in histopathology (*Trautmann et al., 2001*).

*Simon et al., (2006)* estimated epidermal caspase activation in AD skin before and after topical calcineurin inhibitor treatment. They detected not only increased expression of cleaved caspase-3 in keratinocytes of the basal layer but also observed caspase-3 cleavage in one or more layers of the spinous cell layer, in particular in spongiotic areas.

Toxic epidermal necrolysis (Lyell's syndrome) is a severe drug reaction in which the abrupt onset of massive keratinocyte apoptosis results in the detachment of large sheets of epidermis from the underlying
Review of Literature

dermis. It has been shown that this process is associated with highly increased keratinocyte FasL expression, together with conserved levels of keratinocyte Fas expression in vivo (Viard et al., 1998). Functional experiments performed by overlaying cryostat sections of lesional skin with Fas-sensitive cells as targets, has demonstrated that keratinocyte FasL is cytolytically active in TEN. This cytolytic activity could be blocked with monoclonal antibodies that interfere with the interaction of Fas and FasL, thus supporting the hypothesis that increased keratinocyte FasL expression is responsible for the keratinocyte apoptosis that characterizes TEN (Wehrli et al., 2000).

Caproni et al., (2005) investigated whether apoptosis may contribute to the pathogenesis of epidermal changes in dermatitis herpetiformis (DH) and bullous pemphigoides (BP) and, in particular, whether certain apoptosis-related markers such as Bax, Bcl-2, Fas and FasL take part in this process. They found that remarkable increase in the apoptotic rate within the epidermal compartment. Fas showed a prevalently epidermal staining, while Bax/Bcl-2 ratio was almost the same in the epidermis of perilesional/lesional DH, BP and healthy skin specimens.

Schmidt & Waschke (2009) reviewed Apoptosis in pemphigus. They concluded that the apoptotic machinery is involved in pemphigus. The majority of in vitro data indicate that hallmarks of apoptosis, especially changes in nuclear morphology and cell death, are detectable relatively late and follow acantholysis. In later stages of pemphigus pathogenesis, when high serum levels of Fas ligand are present in pemphigus sera, apoptotic cell death may well contribute to lesion
formation. Acantholysis in pemphigus seems to be primarily induced via cell signalling mechanisms including apoptotic caspase signalling.

Choi et al., (2006) examined the involvement of apoptosis in the active fixed drug eruption (FDE) lesions by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labelling (TUNEL) assay and immunohistochemical analysis of caspase-3, Fas and Fas ligand (FasL). The result of the study showed that TUNEL positivity was strongly observed in the basal keratinocytes, and also weakly observed in a few keratinocytes in the granular layer. The distribution of TUNEL positive cells was similar to that of the strong staining of active caspase-3. Fas was found mainly in the keratinocytes and some infiltrated dermal cells, whereas FasL was identified predominantly in the intraepidermal and in some basal keratinocytes. Overlapping expression of Fas and FasL was accompanied by apoptosis in the FDE lesions.

Oishi et al., (1994) investigated the expression of Fas antigen on various skin tissues, and on cultured keratinocyte and human skin squamous cell carcinoma. They found that fas was strongly expressed on the keratinocytes of lichenoid eruptions seen in lupus erythematosus and lichen planus, and on the spongiotic or acanthotic epidermis seen in chronic eczema, adult T-cell leukaemia/lymphoma (ATLL) and atopic dermatitis. Its expression was closely correlated with lymphoid infiltrating cells.

Soini et al., (1994) investigated the expression of p53 in sixty-two skin samples from patients with a variety of benign disorders (20 cases of psoriasis, 14 cases of chronic dermatitis, 11 seborrhoeic keratoses, 11 cases of lichen planus), and seven normal skin samples. They found that
detectable accumulation of p53 is a frequent finding in non-neoplastic skin lesions. The accumulation of p53 is possibly a response to an increased proliferation rate of the keratinocytes in these skin diseases, or alternatively it may be associated with apoptosis.

Nakagawa et al., (1994) analyzed immunohistochemically the expression of the Bcl-2 protein in several keratinocytic (KC) tumors and inflammatory skin disorders to investigate the role of Bcl-2 in the development of benign and malignant skin tumors. The result of the study showed that tissue with chronic dermatitis and psoriasis vulgaris scarcely expressed the Bcl-2 protein while all cases with squamous cell carcinomas showed obvious Bcl-2 protein expression.

Hussein et al., (2004) examined the expression patterns of apoptosis-linked molecules (Bcl-2 and p53) in hyperproliferative keratinocytic lesions (12 squamous cell carcinomas, 11 actinic keratoses, 13 psoriasis vulgaris, eight verruca vulgaris, six chronic dermatitis, five seborrheic keratosis, four lichen planus, three epidermodyplasia verruciformis, two condyloma acuminata, two lichen simplex chronicus, and 10 specimens from normal skin). The results of the study showed that Low level of Bcl-2 and p53 protein expression widely found in normal skin and non-tumorigenic keratinocytic lesions.

Trautmann et al., (2000 a) studied the Role of dysregulated apoptosis in atopic dermatitis. They observed that T cells infiltrating the skin upregulate FasR on KC, rendering them susceptible to apoptosis by IFN-γ and induce apoptosis by FasL expressed on T cell surface. KCs are rendered susceptible to apoptosis when FasR numbers reach a threshold
of approximately 40,000 per KC. The threshold for IFN-γ in the induction of FasR on KCs appears to be in the range of 0.1–1.0 ng/mL.

The question of how vesicle formation is restricted to avoid progressive destruction of the skin is of clinical importance. The defense of the epidermis against destruction could be mediated by the eventual resolution of the underlying inflammatory process and the subsequent reduction in the levels of FasL in the epidermis. The epidermal keratinocytes may also respond to the wave of apoptosis by triggering pro-survival programs. Such pro-survival programs could act by rendering keratinocytes resistant to FasL-dependent apoptosis by, for instance, downregulating the expression of Fas or upregulating the expression of antiapoptotic proteins. Alternatively, the pro-survival programs may not be directed at rescuing the keratinocytes that are targeted by FasL, but instead at increasing the proliferation of basal keratinocytes to replenish the cells lost to apoptosis (Iordanov et al., 2005).

Keratinocyte (KC) apoptosis may be an important mechanism of spongiosis. T cells may induce KC apoptosis, leading to a reduced expression of E-cadherin and disruption of the epidermal barrier. T cell–mediated KC apoptosis is located predominantly in suprabasal epidermal layers, suggesting that antiapoptotic mechanisms protect basal KCs (Meyer et al., 2010).
PATIENTS AND METHODS

Study population:

This study was carried out on 50 patients and 10 healthy controls. The patients were selected from patients attending Dermatology outpatient clinic of Vanderbilt University Hospital, Tennessee State, USA; from February 2010 to February 2011. Patients were divided into 5 groups and each group included ten patients:

1. **Group A: Atopic dermatitis.**

   Patients presenting with acute exacerbations of their disease were enrolled. AD patients had definite AD, diagnosed on the basis of Hanifin and Rajka criteria *(Hanifin & Rajka, 1980)*:

   **Major criteria:** Must have three or more of:
   
   1. Pruritus
   2. Typical morphology and distribution:
      
      Flexural lichenification or linearity in adults
      
      Facial and extensor involvement in infants and children
   3. Chronic or chronically-relapsing dermatitis
   4. Personal / family history of atopy (asthma, allergic rhinitis, dermatitis)

   **Minor criteria:** Should have three or more of:
   
   1. Xerosis
   2. Ichthyosis, palmar hyperlinearity, or keratosis pilaris
   3. Immediate (type 1) skin-test reactivity
   4. Raised serum IgE
   5. Early age of onset
   6. Tendency toward cutaneous infections
7. Tendency toward non-specific hand or foot dermatitis
8. Nipple eczema
9. Cheilitis
10. Recurrent conjunctivitis
11. Dennie-Morgan infraorbital fold
12. Keratoconus
13. Anterior subcapsular cataracts
14. Orbital darkening
15. Facial pallor or facial erythema
16. Pityriasis alba
17. Anterior neck folds
18. Itch when sweating
19. Intolerance to wool and lipid solvents
20. Perifollicular accentuation
21. Food intolerance
22. Course influenced by environmental or emotional factors
23. White dermographism or delayed blanch

2. **Group B: Allergic contact dermatitis.**
   ACD was diagnosed clinically and confirmed by patch testing.

3. **Group C: Irritant contact dermatitis.**
   ICD was diagnosed clinically and confirmed by negative patch test.

4. **Group D: Nummular eczema.**
   NE was diagnosed clinically.

5. **Group E: Dyshidrotic eczema.**
   DE was diagnosed clinically.
Inclusion criteria:
1. Active lesions of any severity
2. Duration of the lesions is less than 6 weeks.
3. Patient is 18 years or older.
4. Male or female of any race and ethnicity
5. Patient has signed the informed consent form.

Exclusion criteria:
1. Patient who is pregnant or lactating.
2. Patients using systemic medications within the last 4 weeks
3. Patients using topical medications within the last week
4. Patients who have received immunotherapy within the last year.
5. Conditions that impact the biopsy procedure, e.g. history of bleeding disorders or known lidocaine allergy.
6. Inability of patient to follow study procedures or documented history of the patient being noncompliant.
7. Patients with infected lesions
8. Patients with AD or CD of the face only
9. Patients with other illnesses that may affect apoptotic markers in the skin, e.g. autoimmune disorders, liver disease, renal disease.

Informed consent was obtained from all subjects, and the study was approved by the Ethical Committee of Vanderbilt University, USA. The study was conducted according to the Declaration of Helsinki Principles.
All the patients and control subjects were subjected to the following:

(1) **History taking:**

A purposely-designed sheet was performed for all patients included in this study, including:

1. Personal history: Name, sex, age, residence, occupation, marital status and special habit.
2. Present history: Onset, duration of lesions and course (progressive, regressive or remissions and exacerbations).
3. Past history of drugs intake: Type and duration of intake.
4. Previous treatments: Systemic or local and duration.
5. Family history: Similar condition & its treatment and other skin and systemic diseases.

(2) **General examination:**

- Complete chest, abdomen, cardiac examinations were done.
- Blood pressure, pulse, and weight were recorded.

(3) **Local examination:**

Sites, morphology, configuration of lesions and presence of vesicles were recorded.

(4) **Laboratory investigation:**

- Fasting blood sugar
- Liver functions tests
- Renal functions tests
- Complete blood picture
- Erythrocyte sedimentation rate
(5) Immunohistologic examination

1. 4mm punch skin biopsies were taken from skin lesions and fixed in 10% neutral buffered formalin, routinely processed and paraffin embedded.
2. All biopsies were submitted to histopathologic evaluation to confirm the diagnosis of spongiotic dermatitis.
3. Immunohistochemical staining for caspase-3, Fas, Bcl-2, NF-κB and p53 antibodies were carried out in dermatology research laboratory, dermatology Department, Faculty of Medicine, Vanderbilt University, USA. Immunohistochemical staining was performed on 4-mm sections of paraformaldehyde-fixed and paraffin-embedded skin biopsies.

The following protocol has been used for immunohistochemical staining:

A. Solutions and Reagents

1. Xylene
2. Ethanol, anhydrous denatured, histological grade (100% and 95%)
3. Deionized water (dH₂O)
4. Vector Hematoxylin QS (Vector Laboratories, Inc., Burlingame, CA, USA)
5. Wash Buffer: 10X Phosphate Buffered Saline (PBS): To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na₂HPO₄) and 2.4 g potassium phosphate, monobasic (KH₂PO₄) to 1 L dH₂O. Adjust pH to 7.4.
6. Antibody Diluent: Bovine serum albumin (BSA) 10% in PBS. All antibodies were used in a dilution of 1: 50.
7. Antigen Unmasking: Tris-EDTA (TE): 10 mM Tris/1 mM EDTA (ethylenediaminetetraacetic acid), pH 9.0.
8. Primary antibodies for:

*Caspease-3 (Cell Signaling Technology, Beverly, MA, USA):

Specificity / Sensitivity:
Cleaved Caspase-3 (Asp175) (5A1) is a rabbit monoclonal antibody that detects endogenous levels of the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to Asp175. This antibody does not recognize full length caspase-3 or other cleaved caspases.

Source / Purification:
Monoclonal antibody is produced by immunizing rabbits with a synthetic peptide corresponding to amino-terminal residues adjacent to Asp175 of human caspase-3.

Storage:
Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C.

*Fas (Cell Signaling Technology, Beverly, MA, USA):

Specificity / Sensitivity:
Fas (C18C12) is a rabbit monoclonal antibody that detects endogenous levels of total human Fas protein.

Source / Purification:
Monoclonal antibody is produced by immunizing rabbits with a synthetic peptide corresponding to residues surrounding Lys259 of human Fas, within the intracellular region.

Storage:
Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C.
*Bcl-2 (R & D systems, Minneapolis, MN, USA):*

**Specificity / Sensitivity:**
Bcl-2 is a goat polyclonal antibody that detects endogenous levels of Bcl-2. No cross reactivity was detected with other family members at physiological levels.

**Source / Purification:**
Polyclonal antibodies are produced by immunizing goats with a synthetic peptide corresponding to the carboxy-terminus of Bcl-2. Antibodies are purified by protein A and peptide affinity chromatography.

**Storage:**
Supplied in Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose. Reconstitute the antibody in 100 μL of PBS containing 0.02% NaN3. Store at −20°C.

*NF-κB (Cell Signaling Technology, Beverly, MA, USA):*

**Specificity / Sensitivity:**
NF-κB p65 (E498) is a rabbit polyclonal antibody that detects endogenous levels of total NF-κB p65/RelA protein. It does not cross-react with other NF-κB/Rel family members.

**Source / Purification:**
Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide corresponding to residues surrounding Glu498 of human NF-κB p65/RelA protein. Antibodies are purified by protein A and peptide affinity chromatography.

**Storage:**
Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA and 50% glycerol. Store at −20°C.
Patients and Methods

*P53 (Santa Cruz Biotechnology, Santa Cruz, CA, USA):*

**Specificity / Sensitivity:**

P53 (DO-2) is a mouse monoclonal antibody raised against amino acids 10-16 of p53 of human origin. P53 antibody detects endogenous levels of total p53 protein. The antibody does not cross-react with other p53-related proteins.

**Source / Purification:**

Monoclonal antibody is produced by immunizing animals with a full-length p53 fused to Maltose Binding Protein (MBP-p53). Antibodies are purified by protein A chromatography.

**Storage:**

Each vial contains 200 μg IgG1 in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin. Store at 4° C.

9. **Biotinylated secondary antibody (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA, USA):**

- Antirabbit biotinylated secondary antibody for caspase-3, Fas and NF-κB.
- Antimouse biotinylated secondary antibody for p53.
- Antigoat biotinylated secondary antibody for Bcl-2.

10. **ABC Reagent (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA, USA):** Prepare according to manufacturer’s instructions 30 minutes before use.

11. **AEC substrate (Vector Laboratories, Inc., Burlingame, CA, USA):** Prepare according to manufacturer’s recommendations.
B. Deparaffinization/ Rehydration

1. **Deparaffinize/hydrate sections:**
   a. After overnight incubation at 37°C, incubate sections in three washes of xylene for 5 minutes each.
   b. Incubate sections in two washes of 100% ethanol for 5 minutes each.

2. Wash sections 3-4 times in dH₂O for 5 minutes each.

C. Antigen Unmasking

3. Bring slides to a boil in 10 mM TE/1 mM EDTA, pH 9.0 then maintain at a sub-boiling temperature for 11 minutes. Cool on the bench for 30 minutes.

D. Staining

4. Add 100-400 μl primary antibody diluted in PBS to each section. Incubate overnight at 4°C in a humidity chamber.

5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.

6. Add 100-400 μl biotinylated secondary antibody, diluted in PBS per manufacturer’s recommendation, to each section. Incubate 30 minutes at room temperature.

7. Prepare ABC reagent according to the manufacturer’s instructions and incubate solution for 30 minutes at room temperature.

8. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.

9. Add 100-400 μl ABC reagent to each section and incubate for 30 minutes at room temperature.

10. Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.
11. Add 100-400 μl AEC to each section and monitor staining closely.
12. As soon as the sections develop, immerse slides in dH₂O.
13. Counterstain sections in hematoxylin.
14. Wash sections in dH₂O two times for 5 minutes each.
15. Dehydrate sections:
   a. Incubate sections in 95% ethanol two times for 10 seconds each.
   b. Repeat in xylene, incubating sections two times for 10 seconds each.

All tissue sections were stained under similar conditions to ensure equal staining quality. Positive and negative controls were stained appropriately in the same settings. Negative control sections were treated in the same way as above, using the strain-specific blocking serum while omitting the primary antibody labeling.

Specimens were examined using a light microscope (Nikon, Optiphot 1, and Japan). Photos were taken by a Nikon digital camera. Specimens were examined with objective magnifications of 10, 20, 40, 100 and 400. Five high power fields were randomly chosen for each section. The cells with clearly defined staining compared with the positive control were counted, and the count divided by the total number of cells in each field. The mean of the five fields was estimated for each sample. Each field was evaluated for the proportion of stained cells and staining intensity.
Evaluation of immunostaining

The site of the staining was determined (cytoplasmic, nuclear or membranous) and the distribution of positive cells was noted. To semiquantitatively evaluate the expression of antibodies, the numbers of positively stained cell layers was evaluated in accordance with the scoring system devised by Liang et al., 2009. Each score represents the mean value of different fields from three sections of each specimen. This system results in a score ranging from 0–3 for both the degree of positivity (percentage of positively stained epidermal cells: 0; < 1%, 1; 1–10%, 2; 10–50%, 3; > 50%) and the degree of intensity of staining [from faint-brown (score 1) to deep-brown (score 3)]. The sum of the two scores was taken as the level of expression. The results were graded into:

Negative expression: if the sum of the two scores is 0.
Weak expression: if the sum of the two scores is 1 or less than 4.
Moderate expression: if the sum of the two scores is 4 or less than 6.
Strong expression: if the sum of the two scores is 6.

Statistical analysis:

Statistical analysis system was used for data management and analysis. The significance of the differences was determined using the statistical package Graph Pad Prism, version 3.02 for Windows (Graph Pad Software Inc., San Diego, CA, USA). Statistical analysis was done according to Benko (2007).

Statistical tests:

Descriptive statistics was presented as mean ± standard deviations for continuous variables and number and percentage for categorical variables. Comparisons between groups were done using a nonparametric
analysis of variance (ANOVA) procedure when indicated. Correlations between numeric variables were measured by Pearson's correlation coefficients. The Student’s two-tailed t-test was also performed to identify the difference in the mean values.

**Significance of results:**

The corresponding P value for each test was directly computed by the microprocessor, in which we used the one call test values:

- Non – significant difference when \( P > 0.05 \)
- Significant difference when \( P < 0.05 \) & \( < 0.01 \)
- Highly significant difference when \( P < 0.001 \)

All P values were two-tailed (**Campbell, 2003**).

**Tabulation and graphical representation:**

Tabulation and graphical representation were done according to **Coggon (2003)**.
RESULTS

Clinical data of the studied groups:

The number of the patients included in this study was 50 patients, while the number of the control group was 10 healthy subjects (Table 12). Patients were divided into five groups (A, B, C, D and E) (Table 7-11). Each group included ten patients.

Table (7): Clinical data of group A

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<th>Bcl-2</th>
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Table (8): Clinical data of group B

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### Table (9): Clinical data of group C

<table>
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<tr>
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<th>Age</th>
<th>Sex</th>
<th>Duration</th>
<th>Caspase-3</th>
<th>Fas</th>
<th>Bcl-2</th>
<th>NF-κB</th>
<th>P53</th>
</tr>
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### Table (10): Clinical data of group D

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<th>Age</th>
<th>Sex</th>
<th>Duration</th>
<th>Caspase-3</th>
<th>Fas</th>
<th>Bcl-2</th>
<th>NF-κB</th>
<th>P53</th>
</tr>
</thead>
<tbody>
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### Results

**Table (11): Clinical data of group E**

<table>
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<th>Age</th>
<th>Sex</th>
<th>Duration</th>
<th>Caspase-3</th>
<th>Fas</th>
<th>Bcl-2</th>
<th>NF-κB</th>
<th>P53</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
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<td>2.7</td>
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</tr>
<tr>
<td>3.</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
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</tr>
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<tr>
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<tr>
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</table>

**Table (12): Clinical data of the control group**

<table>
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<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Caspase-3</th>
<th>Fas</th>
<th>Bcl-2</th>
<th>NF-κB</th>
<th>P53</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>29</td>
<td>Female</td>
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<td>2.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>40</td>
<td>Male</td>
<td>2.3</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
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<td>Male</td>
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<td>0</td>
</tr>
<tr>
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<td>2</td>
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<tr>
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<td>2</td>
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<td>0</td>
<td>2.3</td>
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</tr>
</tbody>
</table>
**Results**

**Demographic characteristics of the studied groups:**

This study included 50 cases with spongiotic disorders (34 males and 16 females) and 10 healthy persons served as control group (7 males and 3 females), *(Table 13).*

**Table (13): Group sex-cross tabulation**

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Patients</td>
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<td>16</td>
<td>50</td>
</tr>
<tr>
<td>Control</td>
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<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>19</td>
<td>60</td>
</tr>
</tbody>
</table>

The mean age of the patients group was 31.56 years ± 8.089 years, while the mean age of the control group was 29.50 years ± 8.127. There was no statistically significant difference between patients group and control group (P-value =0.465), *(Table 14).*

**Table (14): Group age-cross tabulation**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>50</td>
<td>18 y</td>
<td>50 y</td>
<td>31.56</td>
<td>8.089</td>
<td>0.465</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>18 y</td>
<td>43 y</td>
<td>29.5</td>
<td>8.127</td>
<td></td>
</tr>
</tbody>
</table>

*P*-value* equal to or less than 0.05 is significant.

The mean age of the male patients group was 32.88 years ± 8.190 years, while the mean age of the male control group was 28.43 years ± 9.502 years. There was no statistically significant difference between male patients group and male control group (P-value =0.209), *(Table 15).*

The mean age of the female patients group was 28.75 years ± 7.335 years, while the mean age of the female control group was 32.00 years ± 3.606 years. There was no statistically significant difference between
Results

female patients group and female control group (P-value =0.470), (Table 15).

Table (15): Group age –sex statistics

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Male</td>
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<td>8.190</td>
<td>28.43</td>
</tr>
<tr>
<td>Female</td>
<td>28.75</td>
<td>7.335</td>
<td>32.00</td>
</tr>
</tbody>
</table>

y= years, SD=standard deviation
P-value * equal to or less than 0.05 is significant.

AD patients had a mean age of 30.4 years ± 10.574 years, ACD patients had a mean age of 30.79 years ± 8.121 years, ICD patients had a mean age of 29.799 years ± 7.568 years, NE patients had a mean age of 33.799 years ±6.579 years and DE patients had a mean age of 33.0 years ±8.027 years. Using bivariate statistical analysis, there was no statistically significant correlation between age of the patients and cutaneous lesions, (Table 16).

Table (16): Correlation between age of the patients and different patient subgroups

<table>
<thead>
<tr>
<th>Variable</th>
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<th>ACD</th>
<th>ICD</th>
<th>NE</th>
<th>DE</th>
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</thead>
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<td>Minimum</td>
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<td>19</td>
<td>23</td>
<td>23</td>
</tr>
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<td>Maximum</td>
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<td>30.79</td>
<td>29.799</td>
<td>33.799</td>
<td>33.0</td>
</tr>
<tr>
<td>SD</td>
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<td>8.121</td>
<td>7.568</td>
<td>6.579</td>
<td>8.027</td>
</tr>
<tr>
<td>r</td>
<td>0.145</td>
<td>-0.235</td>
<td>0.092</td>
<td>0.251</td>
<td>-0.022</td>
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<tr>
<td>P-value*</td>
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<td>0.513</td>
<td>0.800</td>
<td>0.484</td>
<td>0.950</td>
</tr>
</tbody>
</table>

y= years, SD=standard deviation
r= Correlation
* Correlation is significant at the 0.05 level
Results

The mean duration was 2.500 weeks ± 1.249 weeks. The AD patients had a mean duration of 2.400 weeks ± 1.116 weeks, ACD patients had a mean duration of 2.600 weeks ± 1.174 weeks, ICD patients had a mean duration of 2.200 weeks ± 1.398 weeks, NE patients had a mean duration of 2.900 weeks ± 1.101 weeks and DE patients had a mean duration of 2.400 weeks ± 1.265 weeks, (Table 17).

Table (17): Comparison of the mean value of duration of lesions in different patient subgroups

<table>
<thead>
<tr>
<th>Variable</th>
<th>AD</th>
<th>ACD</th>
<th>ICD</th>
<th>NE</th>
<th>DE</th>
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</thead>
<tbody>
<tr>
<td>Duration</td>
<td>Mean</td>
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<td>2.600</td>
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<td>2.900</td>
</tr>
<tr>
<td>SD</td>
<td>1.430</td>
<td>1.174</td>
<td>1.398</td>
<td>1.101</td>
<td>1.265</td>
</tr>
</tbody>
</table>

SD=standard deviation

The immunopathological changes:

In the present study the mean value of cleaved caspase-3 expression was 2.906 ± 0.855 while it was 0.430 ± 0.909 in the control group, (Table 18) & (Figure 4 & 5). There was a statistically significant difference between patients group and control group (P-value <0.001) in favor of patients group. Cleaved caspase-3 was detectable in keratinocytes of the basal layer of all skin specimens taken from lesional skin as brown cytoplasmic staining. In the spinous cell layer, cleaved caspase-3 was observed in all specimens taken from lesional skin. Strongest positive staining was noticed in areas of spongiosis. In contrast, in normal skin, cleaved caspase-3 staining was rarely detectable.

The mean value of caspase-3 expression in the male patients group was 2.903 ± 0.867 while it was in the male control group 0.328 ± 0.869, (Table 19) & (Figure 6). There was a statistically significant difference
Results

between patients group and control group (P-value <0.001) in favor of males of the patients group.

The mean value of caspase-3 expression in the female patients group was 2.875 ± 0.876 while it was in the female control group 0.666 ± 1.155, (Table 20) & (Figure 7). There was a statistically significant difference between patients group and control group (P-value <0.001) in favor of females of the patients group.

The mean value of caspase-3 expression was 2.903 ± 0.867 in male patients, while it was 2.875 ± 0.876 in female patients. The mean value of caspase-3 expression in male patients is higher than female patients. There was no statistically significant difference between both groups (P-value =0.916), (Table 21 & Figure 8).

When we studied the differences in cleaved caspase-3 expression values among different patient subgroups using Two-Way analysis of variance (ANOVA), there was no statistically significant difference in mean cleaved caspase-3 expression values between different groups (P-value =0.604). The cleaved caspase-3 expression values decreased in the following order: AD > ACD > ICD > DE > NE (3.360 ± 1.129 > 3.070 ± 0.857 > 3.040 ± 0.8656 > 2.630 ± 0.4347 > 2.430 ± 0.6516, respectively) (Table 22) & (Figure 9).

In the present study the mean value of Fas expression was 2.630 ± 0.601 while it was 0.470 ± 1.004 in the control group (Table 18) & (Figure 4 & 10). There was a statistically significant difference between patients group and control group (P-value <0.001) in favor of patients group. Positive Fas expression of keratinocytes was observed in lesional
skin as brown cytoplasmic staining. In contrast, Fas expression on keratinocytes was almost undetectable in normal skin.

The mean value of Fas expression in the male patients group was 2.724 ± 0.644 while it was 0.385 ± 1.021 in the male control group, (Table 19) & (Figure 11). There was a statistically significant difference between patients group and control group (P-value <0.001) in favor of males of the patients group.

The mean value of Fas expression in the female patients group was 2.431± 0.451 while it was 0.666 ± 1.155 in the female control group, (Table 20) & (Figure 12). There was a statistically significant difference between patients group and control group (P-value <0.001) in favor of females of the patients group.

The mean value of Fas expression was 2.724 ± 0.644 in male patients, while it was 2.431 ± 0.451 in female patients. The mean value of Fas expression in male patients is higher than female patients. There was no statistically significant difference between both groups (P-value =0.916), (Table 21 & Figure 13).

When we studied the differences in Fas expression values among different patient subgroups using Two-Way ANOVA, there was no statistically significant difference in mean Fas expression values between different groups (P-value = 0.109). The Fas expression values decreased in the following order: ACD >AD > NE > ICD > DE (2.930 ± 0.978 > 2.560 ± 0.497 > 2.600 ± 0.416 > 2.560 ± 0.476 > 2.500 ± 0.480, respectively) (Table 22) & (Figure 14).
Results

The mean value of Bcl-2 expression was 0.580 ± 0.945 in the patients group while it was 0.470 ± 1.004 in the control group, (Table 18) & (Figure 4). Bcl-2 expression was detected as brown cytoplasmic staining. There was no statistically significant difference between patients group and control group (P-value = 0.740).

The mean value of Bcl-2 expression in males of the patients group was 0.491 ± 0.906 while it was 0.285 ± 0.756 in males of the control group, (Table 19). There was no statistically significant difference between males of the patients group and control group (P-value =0.579).

The mean value of Bcl-2 expression in females of the patients group 0.518 ± 0.930, while it was 0.900 ± 1.559 in females of the control group, (Table 20). There was no statistically significant difference between females of the patients group and control group (P-value =0.562).

The mean value of Bcl-2 expression in male patients was 0.491 ± 0.906, while it was 0.518 ± 0.930 in female patients. The mean value of Bcl-2 expression in female patients is higher than male patients. There was no statistically significant difference (P-value =0.921), (Table 21).

When we studied the differences in Bcl-2 expression values among different patient subgroups using Two-Way ANOVA, there was no statistically significant difference in mean Bcl-2 expression values between different groups (P-value =0.684). The Bcl-2 expression values decreased in the following order: ACD > AD > DE > ICD > NE (0.670 ± 1.095 > 0.630 ± 1.018 > 0.600 ± 0.966 > 0.400 ± 0.843 > 0.200 ± 0.632, respectively) (Table 22).
The mean value of NF-κB expression was 0.372 ± 0.803 in the patients group while it was 0.630 ± 1.018 in the control group, (Table 18) & (Figure 4). NF-κB expression was detected as brown cytoplasmic staining. There was no statistically significant difference between patients group and control group (P-value = 0.379).

The mean value of NF-κB expression in males of the patients group was 0.391 ± 0.864 while it was 0.285 ± 0.755 in males of the control group, (Table 19). There was no statistically significant difference between males of the patients group and control group (P-value =0.766).

The mean value of NF-κB expression in females of the patients group 0.393 ± 0.848, while it was 0.666 ± 1.155 in females of the control group, (Table 20). There was no statistically significant difference between females of the patients group and control group (P-value =0.632).

The mean value of NF-κB expression in male patients was 0.391 ± 0.864, while it was 0.393 ± 0.848 in female patients. The mean value of NF-κB expression in female patients is higher than male patients. There was no statistically significant difference (P-value =0.992), (Table 21).

When we studied the differences in NF-κB expression values among different patient subgroups using Two-Way ANOVA, there was no statistically significant difference in mean NF-κB expression values between different groups (P-value = 0.759). The NF-κB expression values decreased in the following order: AD >ACD > DE >NE> ICD
(0.700 ± 1.139 > 0.660 ± 1.066 > 0.470 ± 1.004 > 0.430 ± 0.909 > 0.200 ± 0.632, respectively) (Table 22).

The mean value of p53 expression was 0.432 ± 0.878 in the patients group while it was 0.400 ± 0.843 in the control group, (Table 18) & (Figure 4). p53 expression was detected as brown nuclear staining. There was no statistically significant difference between patients group and control group (P-value = 0.967).

The mean value of p53 expression in males of the patients group was 0.314 ± 0.777 while it was 0.285 ± 0.755 in males of the control group, (Table 19). There was no statistically significant difference between males of the patients group and control group (P-value =0.977).

The mean value of p53 expression in females of the patients group 0.681 ± 1.047, while it was 0.666 ± 1.155 in females of the control group, (Table 20). There was no statistically significant difference between females of the patients group and control group (P-value =0.995).

The mean value of p53 expression in male patients was 0.314 ± 0.777, while it was 0.681 ± 1.047 in female patients. The mean value of p53 expression in female patients is higher than male patients. There was no statistically significant difference (P-value =0.171), (Table 21).

When we studied the differences in p53 expression values among different patient subgroups using Two-Way ANOVA, there was no statistically significant difference in mean p53 expression values between different groups (P-value =0.298). The p53 expression values decreased in the following order: ACD > AD > NE > DE > ICD (0.700 ± 1.139 >
Results

0.630 ± 1.018 > 0.430 ± 0.909 > 0.222 ± 0.666 > 0.200 ± 0.632, respectively) (Table 22).

Table (18): Comparison between patients group and control group regarding expression of caspase-3, Fas, Bcl-2, NF-κB and p53

<table>
<thead>
<tr>
<th></th>
<th>Patients (50)</th>
<th>Controls (10)</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>2.906</td>
<td>0.855</td>
<td>0.430</td>
</tr>
<tr>
<td>Fas</td>
<td>2.630</td>
<td>0.601</td>
<td>0.470</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>0.580</td>
<td>0.945</td>
<td>0.470</td>
</tr>
<tr>
<td>NF-κB</td>
<td>0.372</td>
<td>0.803</td>
<td>0.630</td>
</tr>
<tr>
<td>p53</td>
<td>0.432</td>
<td>0.878</td>
<td>0.400</td>
</tr>
</tbody>
</table>

SD=standard deviation
P-value * equal to or less than 0.05 is significant.

Table (19): Comparison between male patients group and male control group regarding caspase-3, Fas, Bcl-2, NF-κB and p53

<table>
<thead>
<tr>
<th></th>
<th>Male Patients (34)</th>
<th>Male Control (7)</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>2.903</td>
<td>0.867</td>
<td>0.328</td>
</tr>
<tr>
<td>Fas</td>
<td>2.724</td>
<td>0.644</td>
<td>0.385</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>0.491</td>
<td>0.906</td>
<td>0.285</td>
</tr>
<tr>
<td>NF-κB</td>
<td>0.391</td>
<td>0.864</td>
<td>0.285</td>
</tr>
<tr>
<td>p53</td>
<td>0.314</td>
<td>0.777</td>
<td>0.285</td>
</tr>
</tbody>
</table>

SD=standard deviation
P-value * equal to or less than 0.05 is significant.
### Results

**Table (20): Comparison between female patients group and female control group regarding caspase-3, Fas, Bcl-2, NF-κB and p53**

<table>
<thead>
<tr>
<th></th>
<th>Female Patients (16)</th>
<th>Female Control (3)</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>2.875</td>
<td>0.876</td>
<td>0.666</td>
</tr>
<tr>
<td>Fas</td>
<td>2.431</td>
<td>0.451</td>
<td>0.666</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>0.518</td>
<td>0.930</td>
<td>0.900</td>
</tr>
<tr>
<td>NF-κB</td>
<td>0.393</td>
<td>0.848</td>
<td>0.666</td>
</tr>
<tr>
<td>p53</td>
<td>0.681</td>
<td>1.047</td>
<td>0.666</td>
</tr>
</tbody>
</table>

SD=standard deviation

P-value * equal to or less than 0.05 is significant.

**Table (21): Comparison between male patients and female patients regarding caspase-3, Fas, Bcl-2, NF-κB and p53**

<table>
<thead>
<tr>
<th></th>
<th>Male Patients (34)</th>
<th>Female patients (16)</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>2.903</td>
<td>0.867</td>
<td>2.875</td>
</tr>
<tr>
<td>Fas</td>
<td>2.724</td>
<td>0.644</td>
<td>2.431</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>0.491</td>
<td>0.906</td>
<td>0.518</td>
</tr>
<tr>
<td>NF-κB</td>
<td>0.391</td>
<td>0.864</td>
<td>0.393</td>
</tr>
<tr>
<td>p53</td>
<td>0.432</td>
<td>0.878</td>
<td>0.681</td>
</tr>
</tbody>
</table>

SD=standard deviation

P-value * equal to or less than 0.05 is significant.
Table (22): Comparison between different patient subgroups regarding caspase-3, Fas, Bcl-2, NF-κB and p53

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>ACD</th>
<th>ICD</th>
<th>NE</th>
<th>DE</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase-3 Mean</td>
<td>3.360</td>
<td>3.070</td>
<td>3.040</td>
<td>2.430</td>
<td>2.630</td>
<td>0.604</td>
</tr>
<tr>
<td>SD</td>
<td>1.129</td>
<td>0.857</td>
<td>0.865</td>
<td>0.651</td>
<td>0.434</td>
<td></td>
</tr>
<tr>
<td>Fas Mean</td>
<td>2.560</td>
<td>2.930</td>
<td>2.560</td>
<td>2.600</td>
<td>2.500</td>
<td>0.137</td>
</tr>
<tr>
<td>SD</td>
<td>0.497</td>
<td>0.978</td>
<td>0.476</td>
<td>0.416</td>
<td>0.480</td>
<td></td>
</tr>
<tr>
<td>Bcl-2 Mean</td>
<td>0.630</td>
<td>0.670</td>
<td>0.400</td>
<td>0.200</td>
<td>0.600</td>
<td>0.684</td>
</tr>
<tr>
<td>SD</td>
<td>1.018</td>
<td>1.095</td>
<td>0.843</td>
<td>0.632</td>
<td>0.966</td>
<td></td>
</tr>
<tr>
<td>NF-κB Mean</td>
<td>0.700</td>
<td>0.660</td>
<td>0.200</td>
<td>0.430</td>
<td>0.470</td>
<td>0.759</td>
</tr>
<tr>
<td>SD</td>
<td>1.139</td>
<td>1.066</td>
<td>0.632</td>
<td>0.909</td>
<td>1.004</td>
<td></td>
</tr>
<tr>
<td>p53 Mean</td>
<td>0.630</td>
<td>0.700</td>
<td>0.200</td>
<td>0.430</td>
<td>0.222</td>
<td>0.298</td>
</tr>
<tr>
<td>SD</td>
<td>1.018</td>
<td>1.139</td>
<td>0.632</td>
<td>0.909</td>
<td>0.666</td>
<td></td>
</tr>
</tbody>
</table>

SD=standard deviation

P-value * equal to or less than 0.05 is significant.
Results

Figure (4): Comparison between patients group and control group regarding expression of caspase-3, Fas, Bcl-2, NF-κB and p53.

Figure (5): Comparison of caspase-3 expression between the patients and the controls.
Results

Figure (6): Comparison of caspase-3 expression between the male patients and the male controls.

Figure (7): Comparison of caspase-3 expression between the female patients and the female controls.
Results

Figure (8): Comparison of caspase-3 expression between female and male patients.

Figure (9): Comparison of caspase-3 expression between different patient subgroups.
**Results**

Figure (10): Comparison of Fas expression between the patients and the controls.

Figure (11): Comparison of Fas expression between the male patients and the male controls.
Results

Figure (12): Comparison of Fas expression between the female patients and the female controls.

Figure (13): Comparison of Fas expression between male and female patients.
Figure (14): Comparison of Fas expression between different patient subgroups.

There was no statistically significant correlation between age of patients and expression of caspase-3, Fas, Bcl-2, NF-κB and p53 (r =0.468, P-value=0.001). There was statistically significant correlation between duration of lesions and expression of caspase-3 (r =0.468, P-value=0.001), while there was no statistically significant correlation between duration of lesions and expression of Fas, Bcl-2, NF-κB and p53 (r =0.468, P-value=0.001), (Table 23).
### Results

**Table (23): Correlation between age and duration and caspase-3, Fas, Bcl-2, NF-κB and p53 expression**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Caspase-3</th>
<th>Fas</th>
<th>Bcl-2</th>
<th>NF-κB</th>
<th>p53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>r</td>
<td>-0.036</td>
<td>0.015</td>
<td>0.088</td>
<td>-0.265</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.800</td>
<td>0.917</td>
<td>0.542</td>
<td>0.062</td>
</tr>
<tr>
<td>Duration</td>
<td>r</td>
<td>-0.327</td>
<td>0.023</td>
<td>-0.171</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.020*</td>
<td>0.873</td>
<td>0.233</td>
<td>0.684</td>
</tr>
</tbody>
</table>

*r* = Correlation  
*Correlation is significant at the 0.05 level*

When we studied the correlation between caspase-3, Fas, Bcl-2, NF-κB and p53 expression using Independent-Samples T Test, the only statistically significant correlation was between the expression of caspase-3 and fas (*r* =0.364, *P*-value=0.009), (*Table 24*).

### Table (24): Correlation between caspase-3, Fas, Bcl-2, NF-κB and p53 expression

<table>
<thead>
<tr>
<th>Variable</th>
<th>Caspase-3</th>
<th>Fas</th>
<th>Bcl-2</th>
<th>NF-κB</th>
<th>p53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase-3</td>
<td>r</td>
<td>1</td>
<td>0.364</td>
<td>-0.074</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>-</td>
<td>0.009*</td>
<td>0.608</td>
<td>0.951</td>
</tr>
<tr>
<td>Fas</td>
<td>r</td>
<td>0.364</td>
<td>1</td>
<td>0.156</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.009*</td>
<td>-</td>
<td>0.277</td>
<td>0.342</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>r</td>
<td>-0.074</td>
<td>0.156</td>
<td>1</td>
<td>-0.099</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.608</td>
<td>0.277</td>
<td>-</td>
<td>0.491</td>
</tr>
<tr>
<td>NF-κB</td>
<td>r</td>
<td>0.008</td>
<td>0.137</td>
<td>-0.099</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.951</td>
<td>0.342</td>
<td>0.491</td>
<td>-</td>
</tr>
<tr>
<td>p53</td>
<td>r</td>
<td>0.141</td>
<td>0.020</td>
<td>-0.093</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.326</td>
<td>0.886</td>
<td>0.516</td>
<td>0.432</td>
</tr>
</tbody>
</table>

*r* = Correlation  
*Correlation is significant at the 0.05 level*
Figure (15): Adult flexural atopic dermatitis.

Figure (16): Allergic contact dermatitis on the forearm after the use of diclofenac cream.
Results

Figure (17): Irritant contact dermatitis with a sharp margin over the knee.

Figure (18): Nummular eczema.
Results

Figure (19): Dyshidrotic eczema of the palm.

Figure (20): Representative histological findings of acute spongiotic dermatitis (H&E X10).
Results

Figure (21): Representative histological findings of acute spongiotic dermatitis (H&E X40).

Figure (22): A case of atopic dermatitis showing moderate caspase-3 expression (IHX40).
Results

Figure (23): A case of atopic dermatitis showing weak caspase-3 expression (IHX40).

Figure (24): A case of atopic dermatitis showing weak Fas expression (IHX40).
Results

Figure (25): A case of atopic dermatitis showing weak Bcl-2 expression (IHX40).

Figure (26): A case of atopic dermatitis showing weak NF-κB expression (IHX40).
Results

Figure (27): A case of atopic dermatitis showing weak p53 expression (IHX40).

Figure (28): A case of allegic contact dermatitis showing moderate caspase-3 expression (IHX40).
Figure (29): A case of allergic contact dermatitis showing weak caspase-3 expression (IHX40).

Figure (30): A case of allergic contact dermatitis showing moderate Fas expression (IHX40).
Results

Figure (31): A case of allergic contact dermatitis showing weak Fas expression (IHX40).

Figure (32): A case of allergic contact dermatitis showing weak Bcl-2 expression (IHX40).
Figure (33): A case of allergic contact dermatitis showing weak NF-κB expression (IHX40).

Figure (34): A case of allergic contact dermatitis showing weak p53 expression (IHX40).
Results

Figure (35): A case of irritant contact dermatitis showing moderate caspase-3 expression (IHX40).

Figure (36): A case of irritant contact dermatitis showing weak caspase-3 expression (IHX40).
Results

Figure (37): A case of irritant contact dermatitis showing weak Fas expression (IHX40).

Figure (38): A case of irritant contact dermatitis showing weak Bcl-2 expression (IHX40).
Results

Figure (39): A case of irritant contact dermatitis showing weak NF-κB expression (IHX40).

Figure (40): A case of irritant contact dermatitis showing weak p53 expression (IHX40).
Results

Figure (41): A case of nummular eczema showing moderate caspase-3 expression (IHX40).

Figure (42): A case of nummular eczema showing weak caspase-3 expression (IHX40).
Results

Figure (43): A case of nummular eczema showing weak Fas expression (IHX40).

Figure (44): A case of nummular eczema showing weak Bcl-2 expression (IHX40).
Results

Figure (45): A case of nummular eczema showing weak NF-κB expression (IHX40).

Figure (46): A case of dyshidrotic eczema showing weak caspase-3 expression (IHX40).
Figure (47): A case of dyshidrotic eczema showing weak Fas expression (IHX40).

Figure (48): A case of dyshidrotic eczema showing weak Bcl-2 expression (IHX40).
Results

Figure (49): A case of dyshidrotic eczema showing weak NF-κB expression (IHX40).

Figure (50): A case of dyshidrotic eczema showing weak p53 expression (IHX40).
DISCUSSION

The discovery of apoptosis is one of the most important advances in biologic research of recent years. The concept that cell death is not merely random and chaotic but rather is programmed and regulated sparked imagination and revolutionized biologic thinking. Intensive research unveiled apoptosis as a universal and fundamental process inherent in any nucleated cell and tightly regulated and executed by specific, genetically controlled cellular protein systems. Numerous triggers, either physiologic or disease-related, can trigger the apoptotic program, all ultimately converging into a point at which the cell commits to the death process. Apoptotic cells are converted into clusters of membrane-bound particles that are subsequently engulfed and eliminated by macrophages (Reshef et al., 2010).

Programmed cell death (apoptosis) is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, embryonic development and chemical-induced cell death. Programmed cell death also plays a role in the homeostasis of the normal epidermis as well as in the terminal differentiation of keratinocytes resulting in a cornified layer formed by dead keratinocytes that is finally shed from the skin surface (Candi et al., 2005).

Two major pathways of apoptosis have been characterized: the intrinsic pathway, featuring mitochondrial permeability transition, irreversible membrane depolarization, release of cytochrome c, and activation of caspases, and the extrinsic pathway, featuring
Discussion

transmembrane “death receptors” with respective ligands (e.g., tumor necrosis factor, tumor necrosis factor–related apoptosis-inducing ligand, and Fas ligand) and signal transduction pathways, leading to caspase activation (Reshef et al., 2010).

Spongiotic dermatitis is a broad category of inflammatory skin disease in which spongiosis is the microscopic hallmark. Although spongiosis refers only to serum between keratinocytes, the serous fluid is usually accompanied by exocytosis of inflammatory cells (Elder et al., 2005). In its common forms, including allergic contact dermatitis and nummular dermatitis, spongiosis is usually accompanied by lymphocytes (lymphocytic spongiosis) (Machado-Pinto et al., 1996).

The consensus among many dermatopathologists and a few dermatologists is that the expression eczema should be replaced with the term “spongiotic dermatitis.” Spongiosis refers to the histopathologic changes that underlie most of the so-called eczemas, i.e., edema between the keratinocytes of the stratum malpighii, which gives a spongy appearance to the epidermis. Eczematous dermatitis is a T-cell-mediated inflammatory skin disease, where activated T cells may harm epidermal keratinocytes by direct cell–cell contact or secreted proinflammatory cytokines, accounting for at least some of the features of spongiosis formation, the typical epidermal pathology of eczema (Akiba et al., 2002).

During eczematous skin inflammation, the main constituents of the skin, keratinocytes, play an important role in inducing and shaping the immunological response to environmental stimuli. In eczematous dermatitis, activated dermis- and epidermis-infiltrating T cells target KC
for apoptosis. In turn, damaged KCs respond by secreting inflammatory mediators, thus effecting further recruitment of immunocytes to inflamed skin. Further advances will come from identification of the immunoregulatory mechanisms involved in the pathogenesis of eczematous dermatitis (Kerstan et al., 2009).

The eczematous inflammation is characterized by suprabasal epidermal spongiosis and an inflammatory perivascular upper dermal infiltrate composed mainly of activated T cells. The histological hallmark of eczematous disorders consists of a marked KC pathology. During eczematous inflammation, acantholysis and spongiosis in suprabasal epidermal layers may proceed to vesicle formation. The clinical features are related to increased vascular blood flow (erythema), vascular permeability (edema), the migration of T cells into the dermal compartment (infiltration), epidermal spongiosis (vesiculation) and a release of pruritogenic mediators (itch) (Trautmann et al., 2000 b).

This pattern of skin inflammation can be induced and maintained by a variety of environmental or intrinsic factors (e.g. contact allergens, irritants, infective agents, atopy, filaggrin gene mutations) resulting in clinically more or less specific eczematous diseases. During inflammation, resident structural elements of the skin (e.g. KC, fibroblasts, endothelial cells) tightly interact with cells that are actively recruited from the blood in response to inflammatory stimuli. A complex interaction of numerous chemokines controls the recruitment of T cells from the blood vessels and their migration into the dermal unit (Goebeler et al., 2001).
Although the effector pathways of activated T cells have been intensively studied, the active role of epidermal keratinocytes in induction and maintenance of eczematous dermatitis has been rather neglected. It has been suggested that apoptosis of KC induced by T cells and mediated by CD95 is a crucial event in the transition from activation of the immune system to the manifestation of eczematous dermatitis (Kerstan et al., 2009).

Importantly, inflammation of the dermal-epidermal compartment is driven by the elaborated KC-T cell interactions by means of T cell-derived inflammatory cytokines, IFN-γ and a plethora of immunoregulatory mediators produced by KC. Thus, the local response of KC in concert with the reaction of endothelial cells, T cells, mast cells, eosinophils and dendritic cells finally leads to the characteristic clinical and histological appearance of eczema (Bieber, 2010).

It would be of great interest if the pathogenic steps in the development of spongiosis could be dissected on a molecular level. However, investigations into the differential (and unique) expression of inflammatory mediators in spongiotic dermatitis are difficult to interpret as they have to incorporate a direct comparison between different types of diseases, stage of disease (acute versus chronic) or other skin diseases. Bearing the dynamic course of spongiotic dermatitis, it is therefore crucial to define the stage of disease (acute versus chronic) whenever effector mechanisms are evaluated. Especially, if such knowledge should be translated into specific therapeutic interventions, it may be essential to understand mediators which may be fundamentally different in the acute phase as compared to the chronic phase of spongiotic dermatitis (Kerstan et al., 2009).
Depending on the stage of disease, it would be interesting to know if spongiotic dermatitis shares a common ‘cytokine / chemokine signature’, as it has been suggested for lichenoid dermatoses (e.g. lichen planus, lupus erythematosus). *Wenzel & Tuting, 2008* recently showed that upregulation of CXCR3 ligands predominantly on KC is the common intersection of lichenoid dermatoses, thereby presumably facilitating the recruitment of CXCR3+ and granzyme B+ cytotoxic T cells to the basal epidermal layer where they attack basal KC via the perforin/granzyme B pathway.

The importance of understanding the mechanistic machinery of apoptosis is vital because programmed cell death is a component of both health and disease, being initiated by various physiologic and pathologic stimuli. Moreover, the widespread involvement of apoptosis in the pathophysiology of disease lends itself to therapeutic intervention at many different checkpoints. Understanding the mechanisms of apoptosis and other variants of programmed cell death, at the molecular level provides deeper insight into various disease processes and may thus influence therapeutic strategy (*Elmore, 2007*).

Keratinocyte apoptosis is believed to play an important role in the pathogenesis of eczematous dermatitis, in particular for the formation of spongiosis (*Simon et al.2006*).

The present study investigates changes in the expression level of the apoptosis regulatory proteins caspase-3, Fas, Bcl-2, NF-κB and p53 in skin samples of patients with spongiotic disorders in comparison with control skin samples from healthy subjects, with respect to their role in the apoptotic process.
The present study was conducted on 50 patients and 10 healthy controls. The mean age of patients was 31.56 years ± 8.089 years. AD patients had a mean age of 30.4 years ± 10.574 years, ACD patients had a mean age of 30.79 years ± 8.121 years, ICD patients had a mean age of 29.799 years ± 7.568 years, NE patients had a mean age of 33.799 years ± 6.579 years and DE patients had a mean age of 33.0 years ± 8.027 years.

Apoptosis is regarded as a carefully regulated energy-dependent process, characterized by specific morphological and biochemical features in which caspase activation plays a central role. Cysteine proteases that cleave their target proteins at specific aspartic acids (caspases) are present in the cells as inactive zymogens that must be cleaved to generate free catalytic subunits able to associate and form active heterotetramers (Martinon & Tschopp, 2004).

As caspase-3 is the prime executioner caspase of apoptosis (Lavrik et al., 2005), we investigated the in situ cleavage of caspase-3 in eczematous dermatitis using an mAb that detects cleaved (as surrogate marker for activation), but not full-length, caspase-3. We studied caspase-3 expression because it is a very early and highly specific marker of apoptosis. Unlike the TUNEL assay, it can clearly differentiate between apoptotic and necrotic cells.

In the present study the main value of cleaved caspase-3 expression was 2.906 ± 0.855 while it was 0.430 ± 0.909 in the control group. There was a statistically significant difference between patients group and control group (P-value <0.0001) in favor of patients group.
In this study, we demonstrate that caspase-3 cleavage occurs in keratinocytes of both the basal and the spinous layers of the epidermis in acute eczematous lesions and that particular high levels of cleaved caspase-3 are present in spongiotic areas. In cases with spongiotic vesicle formation, single KCs positive for cleaved caspase-3 were seen in the suprabasal epidermal layers close to the vesicle. These findings support the view that proapoptotic pathways are activated in keratinocytes of patients with spongiotic disorders with predominant lymphocytes contributing to at least some of the clinical and histological features of the disease (Young et al., 2003, Simon et al., 2006, Armbruster et al., 2009). T-cell-mediated apoptosis of single KCs is a key feature of epidermal pathology in acute eczematous dermatitis.

In contrast to the lesional skin, staining of cleaved caspase-3 was weak and restricted to the basal layer in normal skin. These findings are in agreement with previously published work, which suggested that caspase-3 cleavage is limited to the basal layer in normal skin, and it is therefore unlikely that it contributes to the process of epidermal differentiation (Hague et al., 2004, Simon et al., 2006). In contrast, other authors observed apoptotic keratinocytes associated with caspase-3 cleavage in the granular layer only and did suggest a role of apoptosis in epidermal differentiation (Weil et al., 1999). The reason for these discrepancies is unclear at the moment, but different methods used in the different laboratories may account for this problem.

The presence of cleaved caspase-3 in the basal layer of normal epidermis as observed in this study implies an apoptotic function in normal keratinocytes. The epidermis shows a high tissue turnover in which keratinocytes are constantly generated from stem cells and transit
amplifying cells in the basal layer. The role of the transit amplifying cells is to increase the number of differentiating cells. Although terminal differentiating keratinocytes die and are shed from the skin surface, it is likely that apoptosis is involved in regulating the balance between proliferating and differentiating cells in the basal layer of the skin (Simon et al. 2006).

On the contrary, caspase-3 may also participate in the regulation of the cell cycle in these cells. It is possible that the putative increased caspase-3 activity in the basal epidermal layer of patients is still not sufficient to keep the balance between apoptotic and proliferating keratinocytes under these pathologic conditions, because epidermal thickening (acanthosis) is usually associated with eczematous dermatitis (Woo et al., 2003).

Taken together, we demonstrate cleavage of caspase-3 in the spinous cell layer especially in spongiotic areas of acute lesional skin. Apoptosis is suggested to be involved in spongiosis formation and mediated by cleavage of caspace-3. The detection of cleaved caspase-3 in the basal layer of normal skin suggests an apoptotic and/or cell cycle function under physiologic conditions.

Apoptosis of keratinocytes has been shown to be associated with cleavage of E-cadherin, an important component of adherence junctions. Therefore, cleavage of E-cadherin likely contributes to spongiosis formation in acute eczematous dermatitis. Because E-cadherin is a proteolytic target of caspase-3 (Trautmann et al., 2001), the data of our study suggest that active caspase-3 cleaves E-cadherin in eczematous dermatitis. In addition, other desmosomal proteins might be targets of
caspase-3 as well, such as plakophilin and desmplakins, plectin and plakoglobin (Aho, 2004).

In the present study the main value of Fas expression was $2.630 \pm 0.601$ while it was $0.470 \pm 1.004$ in the control group. There was a statistically significant difference between patients group and control group (P-value $<0.001$) in favor of patients group. We observed strong Fas expression of keratinocytes in lesional skin. The ring-like staining pattern suggested that a large proportion of the expressed Fas molecules were located on the surface of these cells. In contrast, Fas expression on keratinocytes was almost undetectable in normal skin. The Fas/FasL system is a key molecular regulator of apoptosis (Choi et al., 2006).

Fas, also known as CD95 antigen, is a 48 kDa transmembrane glycoprotein. It is a member of the nerve growth factor receptor/tumor necrosis factor superfamily. This cell surface molecule mediates receptor-triggered apoptosis. Keratinocytes have been described to increase their sensitivity to undergo Fas receptor-mediated apoptosis following the exposure to IFN-γ, largely due to the induction of the Fas receptor gene (Qin et al., 2001).

T cell-mediated CD95-induced KC apoptosis has been postulated as an important pathogenetic event in spongiosis formation. In this context, apoptosis might severely influence the magnitude and spread of the inflammation from the space of T cell invasion by several mechanisms. The most obvious reason could be that efficient reduction of KC surrounding the epidermis-located inflammatory cells is critical, as proposed (Leverkus & Trautmann, 2006).
CD95 is slightly upregulated on KCs throughout all epidermal layers in eczematous dermatitis as compared with healthy skin (Simon et al., 2006). Thus, differential CD95 expression may basically account for the increased susceptibility of KCs to CD95-mediated apoptosis in eczema, but does not explain the apoptosis resistance of basal KCs. The differential expression of pro- and antiapoptotic factors, which may influence the susceptibility to CD95-mediated apoptosis, might provide an explanation for the restriction of spongiosis to suprabasal epidermal layers. CD95–CD95L interactions were shown to activate NF-κB that is critical for the CD95L-induced activation of inflammatory genes (Armbruster et al., 2009).

CD95L is mainly considered proapoptotic ligand that acts by facilitating recruitment of the adaptor protein Fas-associated death domain protein (FADD) and activation of the initiator caspases 8 and 10 to form a “death-inducing signaling complex,” the so-called DISC. Activated initiator caspases process and activate effector caspases, which ultimately cause cell death (Diessenbacher, 2008).

Activated T cells inducing keratinocyte apoptosis by a Fas-mediated pathway have been identified in both contact dermatitis and atopic dermatitis. Early, there is widening of the intercellular spaces with stretching of the desmosomes, i.e., spongiosis, caused by the progressive accumulation of fluid. The pattern seen most commonly is of acute spongiotic dermatitis, although it can be subacute and chronic as well (Trautmann et al., 2000 b, Simon et al., 2006).

Apoptosis of the keratinocytes that is Fas-mediated would occur predominantly from cytotoxicity of the infiltrating T cells and, in part, be
self-induced by keratinocytes. A recent study has indicated that in a stressful situation, keratinocytes could initiate apoptosis by translocating their own intracellular FasL to the cell surface where Fas is present (Viard-Leveugle et al., 2003). However, self-induced apoptosis of keratinocytes is likely to play but a minor role in the pathogenesis of spongiotic dermatitis, in regard to the histopathological features of lesions in which a lymphocyte-mediated attack on epidermal cells is primary event leading to the tissue injury.

Although, it has been reported that apoptosis and its related mediators could play a role in the pathogenesis of AD and ACD, the available few reports could not clarify the exact role of apoptosis and its related mediators in the pathogenesis of other causes of spongiotic dermatitis. To the best of our knowledge, no previous studies have reported the potential role of Bcl-2, NF-κB and p53 markers in the pathogenesis of spongiotic dermatitis.

In the present study we used immunohistochemical staining for Bcl-2 to enable a possible regulatory role to be investigated. Bcl-2 expression was absent or weak in basal and suprabasal cells in lesional skin. The mean bcl2 was 0.580 ± 0.945 in the patients group while it was 0.470 ± 1.004 in the control group. A higher Bcl-2 expression was detected in patients than controls; however, there was no statistically significant difference between patient group and control group (P-value = 0.740). This increase in epidermal Bcl-2 expression with its antiapoptotic effect may explain the increased epidermal thickness in patients with eczematous dermatitis. Proteolytic activation of caspase can be modulated by the suppressing oncoprotein family Bcl-2 (Abdel-Latif et al.2008). On this basis, the lack of keratinocyte expression of Bcl-2
observed in our study could make keratinocyte cell death possible through caspase cascade activation.

Bcl-2 expression in inflammatory diseases has been minimally evaluated and the evaluation of its expression in skin diseases has been largely restricted to cutaneous neoplasms. This polypeptide has been described in benign nevi, melanomas, blue nevi, Spitz nevi, basal cell carcinomas, Bowen’s disease, and Merkel cell carcinomas (Morales-Ducret et al., 1995). Marzano et al., 2007 evaluated the expression of Fas, Bcl-2 and Bax in erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis. They found strong expression of Bcl-2 only in the basal layer. In contrast, Batinac et al., 2007 examined the expression of pro-apoptotic (Bak, Bax) and anti-apoptotic (Bcl-2, Bcl-X) Bcl-2 family of proteins in psoriasis and they found a decrease in Bcl-2 expression in involved psoriatic skin.

In the present study we used also immunohistochemical staining for NF-κB. The mean NF-κB was 0.372 ± 0.803 in the patients group while it was 0.630 ± 1.018 in the control group. There was no statistically significant difference between patient group and control group (P-value = 0.379). only cytoplasmic staining was detected and This is matched with the fact that NF-κB in unstimulated resting cells is restricted to the cytoplasm bound to I kappa B (IkB) that subsequently prevents it from entering the nucleus. When these cells are stimulated, specific kinases phosphorylate IkB causing its rapid degradation by proteosomes with release of NF-κB and its passage into the nucleus.

NF-κB is one of the transcriptional factors that play a critically important role in regulation of cell cycle as well as influencing cell death.
Discussion

pathways in addition to its widely recognized role as a key regulator of immune and inflammatory responses. In skin, NF-κB is proposed to protect epidermis against apoptosis by enhancing the expression of anti-apoptotic factors (Seitz et al., 2000). The effects of NF-κB on apoptosis have far-reaching consequences for normal development and/or homeostasis in many cells and tissues, including the immune system, hair follicles and epidermal appendages (Hussein et al., 2004).

The dysregulation of NF-κB is suggested to play an important role in skin pathology, including proliferative disorders, e.g. psoriasis (Danning et al., 2000), inflammatory processes such as incontinentia pigmenti (Karin & Ben-Neriah, 2000), sunburn (Simon et al., 1994) and autoimmune diseases, as well as in skin carcinogenesis (Bell et al., 2003).

In the present study we used also immunohistochemical staining for p53. In the present study the mean p53 was 0.432 ± 0.878 in the patients group while it was 0.400 ± 0.8433 in the control group. There was no statistically significant difference between patient group and control group (P-value = 0.967). Under normal conditions there is no or minimal p53 expression in sun-protected skin. In the present study all skin samples were obtained from sun-protected skin.

p53 is a tumor suppressor recessive gene located on the short arm of chromosome 17, which plays a role in modulating gene transcription (gene recognition and copying), policing cell cycle checkpoints, activating apoptosis, controlling DNA replication and repair, maintaining genomic stability and responding to genetic insults. p53 has a prominent role in cell response to DNA damage, causing the proliferating cells to
enter G1 arrest. At this pause, cell decides whether to repair the DNA damage (allowing it to re-enter the cell cycle) or commit suicide (if the damage is too great) (Raskin, 1997).

p53 expression in skin diseases has been largely evaluated in relation to UV-induced DNA damage. UV-induced DNA damage activates the mechanisms for removal of DNA damage, delay in cell cycle progression, DNA repair, or apoptosis by transcriptional activation of p-53 related genes, such as p21 and bax. Several studies have shown that UVB also induces mutations in p53 gene. The induced mutations are unique for UVB radiation and are not commonly induced by other carcinogens. Mutations in the p53 gene have been detected in 50% of all human cancers and in the majority of skin carcinomas (El-Domyati et al. 2003).

A high frequency of p53 mutation was reported in pre-malignant actinic keratosis lesions that is considered to be pre-squamous cell carcinoma, and in Bowen's disease that is considered to be in situ squamous cell carcinoma (SCC) of the skin. The majority of these mutations were characteristic UVB mutations and these findings suggested that p53 mutations might be involved in the malignant conversion of precancerous lesions to SCC. Several studies have demonstrated the continued and discontinued regimens of chronic UVB treatment to ultimately result in skin tumor development with 100% incidence, although the kinetics of tumor occurrence is delayed in the latter. Thus, these studies suggest that skin cancer development can be delayed but not stopped with further avoidance of UV exposure (Tomas, 2009).
In conclusion, the results of the present study suggest that KC apoptosis is the initiating event in the development of the epidermal pathology seen in spongiotic dermatitis. Most notably, KC apoptosis occurs in suprabasal cells, where spongiosis and acantholysis take place. Apoptosis of individual KC is the first event leading to disruption of epidermal continuity and vesicle formation. Damage to KCs leads to the loss of intercellular cohesion (acantholysis) and subsequent cleft formation. Fluid influx from the dermis and intercellular edema contributes to spongiosis. The knowledge of this molecular basis is pivotal in understanding the development of pathology in spongiotic disorders, and opens a future for more focused therapeutic applications. Further genetic studies will be needed to confirm these findings and get benefit from both caspase-3 and Fas targeted therapies in the treatment of spongiotic dermatitis. Bcl-2, NF-κB and p53 play no role in the KC apoptosis occurring in spongiotic dermatitis.

To the best of our knowledge, this study is the first one that investigates the relation between apoptosis and spongiosis in five spongiotic disorders (AD, ACD, ICD, NE and DE). This is also the first study that evaluates the expression of five apoptotic markers in spongiotic dermatitis (caspase-3, Fas, Bcl-2, NF-κB and p53).
Apoptosis is a major mechanism of programmed cell death used by to eliminate superfluous and irreparably damaged cells. It has a crucial role in shaping organs during development and controls homeostasis and integrity of tissues throughout life. Apoptosis can be triggered by a wide variety of stimuli, including developmental cues, severe cellular stress or damage to essential cellular components, caused by heat shock, radiation, cytotoxic drugs, infection and oncogenic transformation.

Apoptosis induction occurs through two distinct pathways: intrinsic and extrinsic. The intrinsic pathway is activated by intracellular events and depends on the release of proapoptotic factors from the mitochondria. The extrinsic pathway receives signals through the binding of extracellular protein ligands to proapoptotic death receptors (DRs), located on the cell surface. Both pathways lead to hierarchical activation of specialized proteases called caspases. Apoptotic signals first activate initiator caspases, including caspase-2, -9 and -10. Once stimulated, initiator caspases proteolytically activate the downstream effector caspases, including caspase-3, which in turn cleave numerous essential cellular proteins, thereby leading to the unique morphological and biochemical features of apoptosis, such as plasma membrane ‘blebbing’, cell shrinkage, chromatin condensation and DNA fragmentation.

Apoptosis is regulated by numerous genes and factors such as Fas, TNF, perforin / granzyme B, Bcl-2, NF-κB and p53. Aberrant regulation of apoptotic cell death mechanisms is an important pathological factor in variety of major human diseases. Deficiency in apoptosis is one of the key hallmarks of cancer and also contributes to certain autoimmune
Summary and Conclusion

diseases and metabolic disorders. In contrast, excessive apoptosis is an important component in neurodegenerative disorders, infertility and inflammatory diseases.

Keratinocyte apoptosis is believed to play an important role in the pathogenesis of spongiotic dermatitis, in particular for the formation of spongiosis. The present study investigates changes in the expression level of the apoptosis regulatory proteins caspase-3, Fas, Bcl-2, NF-κB and p53 in skin samples of patients with spongiotic disorders.

This study was carried out on 2 groups:
(1) Patients group that included 50 patients divided into five subgroups:
   - Group (A): atopic dermatitis
   - Group (B): allergic contact dermatitis
   - Group (C): irritant contact dermatitis
   - Group (D): nummular eczema
   - Group (E): dyshidrotic eczema
(2) Control group that included 10 healthy subjects.

All studied individuals were subjected to history taking and clinical examination; we investigated expression of apoptotic regulatory molecules with variable parameters including duration of cutaneous lesions and age of the patients.

The result of this work showed the following:

1. Caspase-3 cleavage occurs in keratinocytes of the spinous layers of the epidermis in acute spongiotic lesions and that particular high levels are present in spongiotic areas.
Summary and Conclusion

2. Positive Fas expression of keratinocytes in acute spongiotic lesions. The ring-like staining pattern suggested that a large proportion of the expressed Fas molecules were located on the surface of these cells.

3. Bcl-2 expression was absent or weak in suprabasal cells in lesional skin. This decrease in epidermal Bcl-2 expression with its antiapoptotic effect may explain the increased sensitivity of keratinocytes to apoptotic stimuli.

4. NF-κB expression was absent or weak in suprabasal cells in lesional skin. This decrease in epidermal NF-κB expression with its antiapoptotic effect may also explain the increased sensitivity of keratinocytes to apoptotic stimuli.

5. P53 expression was absent or weak in suprabasal cells in lesional skin. This indicates that p53 has no role in keratinocytes apoptosis that occurred in spongiotic dermatitis.

Conclusion:

KC apoptosis is the initiating event in the development of the epidermal pathology seen in spongiotic dermatitis (in studied diseases). Most notably, KC apoptosis occurs in suprabasal cells, where spongiosis takes place. Apoptosis of individual KC is the first event leading to disruption of epidermal continuity and vesicle formation. Damage to KCs leads to the loss of intercellular cohesion (acantholysis) and subsequent cleft formation. Fluid influx from the dermis and intercellular edema contributes to spongiosis. The knowledge of this molecular basis is pivotal in understanding the development of pathology in spongiotic disorders, and opens a future for more focused therapeutic applications.
1. The study was done on 50 patients with spongiotic disorders, and it is recommended that similar studies have to be done on larger number of patients.

2. More studies are needed, to evaluate apoptosis in other diseases with spongiosis.

3. More studies are needed, to study the expression of other apoptotic markers in different spongiotic disorders.

4. More studies are needed, to evaluate apoptosis in spongiotic disorders using other methods such as TUNEL method and TEM.

5. More studies are needed, to evaluate the relation between the density and type of inflammatory infiltrate and the extent of apoptosis.

6. More studies are needed, to study treatment of spongiotic disorders by the use of antiapoptotic drugs and the newly developing ones that disrupt the processes mediated by caspase-3 and Fas.
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الملخص العربي

يعتبر الابوتوبوزيس آلية رئيسية لموت الخلايا المبرمج والتي تستخدم للقضاء على الخلايا الزائدة أو الخلايا التالفة التي لا يمكن إصلاحها.

للابوتوبوزيس دور حاسم في تشكيل الأعضاء خلال مراحل التطور وتحقيق التوازن وسلامة الأنسجة في جميع مراحل الحياة.

يمكن حدوث الابوتوبوزيس من قبل مجموعة واسعة من المحفزات، بما في ذلك اشارات النمو، والإجهاد الشديد للخلايا أو الأضرار التي تلحق بالمكونات الأساسية للخلايا والتي تحدث نتيجة الصدمة الحرارية، الإشعاع، الأمراض الكيميائية السامة للخلايا، التحول الورمي والعدوى.

حدث الابوتوبوزيس من خلال مسارين مختلفين: المسار الداخلي و المسار الخارجي.

يتم تنشيط المسار الداخلي عن طريق مثمرات داخل الخلايا ويعتمد على اصدار العوامل المحفزة للابوتوبوزيس من الميتوكوندريا. أما المسار الخارجي فيتم تنشيطه عن طريق ارتباط بعض البروتينات خارج الخلية مع مستقبلات الموت المبرمج التي تقع على سطح الخلية. يؤدي كلا من المسارين إلى تشغيل المنشول لانزيمات بروتيز مخصصة تسمى كاسباس.

تقوم إشارات الابوتوبوزيس أولاً بتشغيل كاسباس البادية، بما في ذلك كاسباس-9 و كاسباس-2 و-10. وبعدها تنشيط كاسباس الناجم، أو كاسباس البادية الفعالة، بما في ذلك كاسباس-9، والتي تؤدي إلى تؤدي إلى تنشيط العديد من البروتينات الخلوية الحيوية وهذا يؤدي إلى التغيرات الشكلية والبيوكيميائية المميزة للابوتوبوزيس مثل تكون فقاقي في غشاء البلازما ، انكسام الخلية، تكيل الكروممات و تجذئة الحاض الحاضر النووي.

تم تنظيم الابوتوبوزيس عن طريق العديد من الجينات والعوامل مثل فاس، عامل تنكر الورم، بيرفوريون/جرانزم ب ، NF-kB ، Bcl-2 و p53. التنظيم الشاذ للابوتوبوزيس يعتبر عملاً مهما في العديد من الأمراض. فان الابوتوبوزيس في الخلايا من السمات الأساسية المميزة في حدوث السرطان ويساهم أيضاً في بعض أمراض المناعة الذاتية واضطرابات الأيض. في
المخصص العرabi

المقابل، موت الخلايا المبرمج المفرط هو عنصر هام في اضطرابات الإصعاب، ونقص تروية القلب، العقم والأمراض الألتهابية.

يعتقد أن موت الخلايا المبرمج للخلايا الكيراتينية يلعب دورا هاما في نشوء الأمراض ذات الانتقال الأسفسجي، ولا سيما لتشكيل الانتقال الأسفسجي.

هدفت هذه الدراسة إلى تحديد مستوى التعبير للبروتينات التنظيمية للإيوببتوزيس في عينات الجلد من المرضى الذين يعانون من الأمراض ذات الانتقال الأسفسجي، وهي كاسباس-3 المنقسم، فاس، NF-κB، Bcl-2، وp53.

تمت هذه الدراسة على مجموعتين:

1. مجموعة المرضى: 50 مريضا مقسمة إلى خمس مجموعات:
   - مجموعة (A) على الحالات التي تعاني من الاكزيميا الوراثية
   - مجموعة (B) على الحالات التي تعاني من الاكزيميا التالاميسية الناتجة من الحساسية للإيوببتوزيس
   - مجموعة (C) على الحالات التي تعاني من الاكزيميا التالاميسية الناتجة من الإثارة للإيوببتوزيس الموضعية
   - مجموعة (D) على الحالات التي تعاني من الاكزيميا المستمرة
   - مجموعة (E) على الحالات التي تعاني من الاكزيميا العرقية

2. المجموعة الضابطة: 10 من الإصحاء.

قد شملت الدراسة أخذ التاريخ المرضي، وفحص السريري، وقد درسنا مستوى كاسباس-3 المنقسم، فاس، NF-κB، Bcl-2، وp53 مع عوامل متغيرة وهي عمر المريض ومدة الإصابات الجلدية.

كانت نتائج الدراسة كالآتي:
1. حدوث انقسام للكاسباس-3 في الخلايا الكيراتينية من طبقات الشائكة في البشرة في الإصابات الأسفسجية الحادة وخصوصا في الأممآلك ذات الانتقال الأسفسجي.
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2. التواجد الالاجيبي للفاس في الخلايا الكيراتينية في الاصابات الاسفنجية الحادة ويرجع نمط التصبغ شبه الحلقي أن تقع نسبة كبرى من جذورات فاس على سطح هذه الخلايا.

3. غياب أو ضعف تواجد ال Bcl-2 في الخلايا فوق القاعدية في الاصابات الجلدية وقد يفسر ضعف تواجد ال Bcl-2 بتأثيره المضاد للالوبوتوزيس زيادة حساسية الخلايا الكيراتينية لمحفزات الالوبوتوزيس.

4. غياب أو ضعف تواجد ال NF-κB في الخلايا فوق القاعدية في الاصابات الجلدية وقد يفسر ضعف تواجد ال NF-κB بتأثيره المضاد للالوبوتوزيس أيضا زيادة حساسية الخلايا الكيراتينية لمحفزات الالوبوتوزيس.

5. غياب أو ضعف تواجد ال p53 في الخلايا فوق القاعدية في الاصابات الجلدية. هذا يدل على أن p53 ليس له دور في موت الخلايا الكيراتينية المبرمج التي تحدث في الأمراض ذات الالتهاب الاسفنجي.

خلصت هذه الدراسة إلى أن موت الخلايا الكيراتينية المبرمج هو الحدث الأول في التغيرات المرضية في الامراض ذات الالتهاب الاسفنجي ويفيد غالبا في الخلايا فوق القاعدية حيث يحدث الالتهاب الأسفنجي. موت الخلايا الكيراتينية المبرمج هو الحدث الأول الذي يؤدي إلى انقطاع استمرارية البشرة وتكوين الجروح. يؤدي تلف الخلايا الكيراتينية إلى فقدان التماشى بين الخلايا (انحلال) وتحدوث التشققات. تتفق السوائل من الأذمة وحذث التورم بين الخلايا يساهم في تكوين الالتهاب الأسفنجي. يعتبر معرفة هذا الأسس الجزيئي محوري في فهم طريقة نشوء الأمراض ذات الالتهاب الأسفنجي، ويفتح مستقبلًا لمزيد من التطبيقات العلاجية المركزية.
وصف موت الخلايا المبرمج (الابوبتيوزيس) في الامراض ذات الانتفاخ الأسفينجي

رسالة مقدمة
توطنة للحصول على درجة الدكتوراه في الأمراض الجلدية وطب وجراحة الجلدية من الطبيب
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