Introduction

Leukemia:

Is cancer of the blood and develops in the bone marrow The bone marrow is the soft, spongy center of certain bones that produces the three major blood cells: white blood cells to fight infection; red blood cells that carry oxygen; and platelets that help with blood clotting and stop bleeding. When a child has leukemia, the bone marrow, for an unknown reason, begins to make white blood cells that do not mature correctly, but continue to reproduce themselves. Normal, healthy cells only reproduce when there is enough space for them to fit.

There are different types of leukemia. According to the American Cancer Society, acute lymphocytic leukemia (ALL) is the type of leukemia that most commonly affects children, most often between the ages of 2 and 4 years. Acute myelogenous leukemia (AML) is the second most common form of leukemia in children. AML generally occurs by the age of 2 years, and is not often seen in older children until the teenage years**.** ALL is cancer of the white blood cells, the cells that normally fight infections. In patients with ALL, the bone marrow produces excess immature white blood cells, called lymphoblasts which are unable to help the body fight infections.

The body can regulate the production of cells by sending signals when to stop. With leukemia, these cells do not respond to the signals to stop and reproduce, regardless of space available.

L-asparaginase(ASNase) has been an element in the treatment for acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma since the late 1960s and remains an essential component of their combination chemotherapy.

L-asparaginase primarily targets malignant lymphoblasts by depletion of the external sources of asparagines, through hydrolysis of asparagines to aspartic acid and ammonia , given the most malignant cells have limited asparagines synthetase activity, the resulting lacks of asparagines leads to apoptosis and malignant cell deaths. Because of unknown risk factors acute pancreatitis occurs in patients receiving ASNase leading to stop treatment with L-asparaginase.

In this research we assessed the effect of ASNase on pancreatic acinar cells both histologically and biochemically and then investigated the preventive effects of the drug (Octreotide) one of the synthetic somatostatin analogue as a prophylactic drug against ASNase –induced pancreatic injury in rats.

Octreotide (SM201-995, Sandostatin):

Somatostatin and octreotide were suggested for the treatment of AP on the basis of their physiopharmacologic properties. Somatostatin is a 14 amino acid peptide that was accidentally discovered in sheep hypothalamus during a search for growth hormone–releasing factor.

Somatostatin is an inhibitor of growth hormone, which also inhibits gastric, pancreatic, biliary, and intestinal secretion. Gastrointestinal motility and splanchnic blood flow are also inhibited by somatostatin. Because of its very short half-life (approximately 2 minutes), somatostatin should be administrated for clinical use in continuous intravenous infusion. In addition to its use in the management of acromegaly, somatostatin or its analogue,octreotide (SMS 201-995, Sandostatin), has been used in the treatment of acute pancreatitis, pancreatic and enterocutaneous fistulas, pancreatic pseudocysts (Gullo&Barbara,1991).

Octreotide was introduced in the early 1980s and offers several advantages over somatostatin, such as a much longer half-life and the options of either subcutaneous or intravenous administration .Octreotide is a powerful inhibitor of exocrine pancreatic secretion and cholecystokinin production. Recently, we demonstrated that prior treatment with octreotide prevented the development of ASNase-induced pancreatic injury in a rat model (Suzuki *et al*.,2008).

In our experimental study we prove the Octreotide ameliorate the damage of pancreatic a cinar cells in induced by L-asparaginase drug.

Chapter 2

Literature Review

One of the primary drugs used in the treatment of ALL is L-asparaginase (ASNase) from E. coli, which has been in clinical use since 1967. (Hill et al.,1967).

2.1 Historical development:

The pioneer observation that turned out to be important for the development of Lasparaginase as a potential antineoplastic agent was made by(Clementi,1922) in1922 revealing the presence of high activity of L-asparaginase in the serum of guinea pig.

High L-asparaginase activity was observed only in guinea pig serum, whereas other mammals were found devoid of this enzyme In1953, Kidd described the regression of transplanted lymphomas in mice and rats by the administration of guinea pig serum. This cytotoxic activity was not present in horse or rabbit serum Neuman and McCoy Neuman&MaCoy,(1956) extended these observations in 1956,they demonstrated that the growth of cell line derived from Walker carcinosarcoma required L-asparagine. Haley et al. in 1961 obtained similar results, using a mouse leukemia cell line. It was Broome in 1961 while working in Kidd's laboratory, who compared Kidd's finding of growth inhibition with the earliest observation by Clementi, and succeeded in concluding that the anti -lymphoma activity in guinea pig Haley et al. in 1961 obtained similar results, using a mouse leukemia cell line. It was Broome in 1961 while working in Kidd's laboratory, who compared Kidd's finding of growth inhibition with the earliest observation by Clementi, and succeeded in concluding that the anti -lymphoma activity in guinea pig sera was due to L-asparaginase .Further investigations of the same author confirmed its therapeutic potential Yellin and Wriston in 1966, succeeded in partial purification of two isoforms of L-asparaginase from the serum of guinea pig.

The first efficacy studies were performed with guinea pig L-asparaginase, but bulk preparation of the enzyme turned out not to be feasible. Although L-asparaginase has been found in various plant and animal species, but due to the difficult extraction procedure of this enzyme, other potential sources like microorganisms were searched. Microorganisms have proved to be very efficient and inexpensive sources of this enzyme. A wide range of microbes comprising bacteria, fungi, yeast, actinomycetes and algae are very efficient producers of this enzyme, but enzyme

properties vary from organism to organism. It has only been produced in large quantities from two bacterial species, *viz. E. coli* and *Erwinia caratovora*Mashburn and Wriston, in 1964 and Campbell and Mashburn in 1969 reported the purification of *E. coli* L-asparaginase, and demonstrated its tumoricidal activity similar to that of guinea pig sera . These findings provided a practical base for large-scale production of enzyme for pre-clinical and clinical studies . Oettgen et al. in 1967 were first to show the efficacy of L-asparaginase in humans with leukemia. Today, L-asparaginase used in the clinic is available in three preparations: two unmodified or native forms, purified from bacterial sources, and one form modified from one of the native preparations. The native preparations are derived from *E. coli* (marketed commercially by Merck & Co. as Elspar), and *Erwinia caratovora* (available as Erwinia L-asparaginase).

AThird preparation, PEG-L-asparaginase (non-proprietary name pegasparaginase), is a chemically modified form of the enzyme, in which native *E. coli* L-asparaginase has been covalently conjugated to PEG. PEG-L-asparaginase, now referred to as pegasparaginase or pegasparagase, was developed in the 1970s and 1980s and was subjected to clinical trials in the1980s. Pegaparaginase (available commercially as Oncaspar) is approved by the Food and Drug Administration for use in combination chemotherapy for the treatment of patients with ALL who are hypersensitive to native form of *E. coli* L-asparaginase. (Pieters and Carroll, 2008).

2.2 Mechanism of action of L- asparaginase:

L-Asparaginase is an important chemotherapeutic agent used for the treatment of a variety of lymphoproliferative disorders and lymphomas, (ALL) in particular. It has been a mainstay of combination chemotherapy protocols used in treatment of pediatric ALL for almost 30 years,(Hann et al.,2000). Based on this, it has also been included in most contemporary, multi-agent regimens for adult ALL (Gokbuget Hoelzer ,2002). L-Asparaginase as a drug has demonstrated effectiveness in induction and subsequent phases of various chemotherapeutic strategies. The major limitation to the use of L-asparaginase is dose limiting clinical hypersensitivity, which develops in 3–78% of patients treated with unmodified forms of enzyme.

Over the last 10 years, PEG-L-asparaginase as an alternate form of L-asparaginase seems to have redressed the problems being faced with the native preparations (Ashihara et al.,1978;Park et al.,1981) .

Tumor cells, more specifically lymphatic cells, require huge amount of asparagines to keep up with their rapid malignant growth. This means they use both asparagine from the diet (blood serum) as well as what they can make themselves (which is limited) because leukemic lymphoblasts and certain other tumor cells have very low levels ofL-asparagine synthetase to satisfy their large demand of asparagines .

L-asparaginase as a drug exploits this unusually high requirement tumor cells have for the amino acid asparagines. L-asparaginase catalyses the hydrolysis of L-asparagine to L-aspartic acid and ammonia(Fig2.1.)L- Asparaginaseresults in depletion of serum asparagine and kills tumor cells by depriving them of an essential factor required for protein synthesis . Healthy cells however escape un affected, as they are capable of synthesizing asparagine themselves with the help of the enzyme L-asparagine synthetase which is present in sufficient amounts, Asselin et al.,(1993) have quantified cell kill bothin vitroandin vivoin patients with ALL under going treatment with L-asparaginase as a single agent. Cell cycle arrest in the G1 phase has been reported in the murine L5178Y cell line and the MOLT-4 human T-lymphoblastoid line (Shimizu et al.,1992), resulting in apoptosis. A human acute lymphoblastic leukemia cell line is markedly inhibited by asparaginases, the effect being 10-fold higher for Erwinia caratovora L-asparagines.

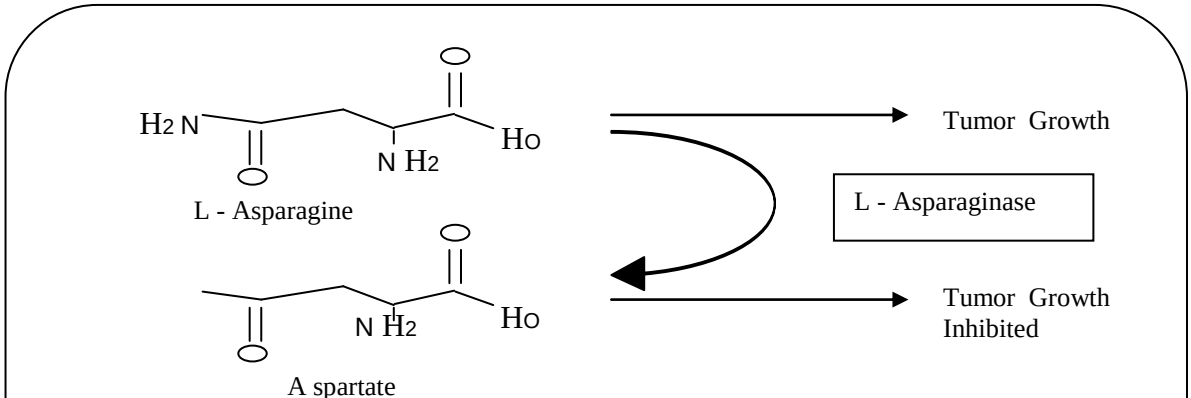


Fig.(2.1):Mechanism of Action of L-asparaginase

(www.elsevier /locate/ citrevone**.)**

The mode of asparaginase action in anti-cancer therapy is complicated by the observation that asparaginase preparations also possess glutaminase activity, rapidly reducing circulating concentrations of glutamine in the plasma of patients .Glutamine deamination values are highly correlated with serum asparaginase activity (Grigoryan et al., 2004). Several reports suggest that the cytotoxic effects of asparaginase are related to reductions in cellular glutamine. Preclinical testing of a novel, glutaminase-free form of asparaginase (isolated from the *Vibrio succinogenes* microbe, subsequently reclassified as *Wolinella succinogenes*) found this enzyme to retain anti-lymphoma properties while lacking hepatotoxicity and immunosuppressive actions (Durden et al., 1983 ; Distasio et al., 1982,. These studies suggest that depletion of glutamine may be one reason for asparaginase toxicity.

Bunpo et al.,(2008) on their studies on normal mice lymphocytes in the spleen, thymus and bone marrow demonstrated that Asparaginase depletes circulating and intracellular glutamine,inhibiting cellular growth and reducing protein synthesis atthe level of mRNA translation initiation in spleen and other tissues or cell types. These responses were not present when mice were treated with a virtually glutaminase-free asparaginase isolated from the *Wolinella* (*Vibrio*) *succinogenes*microbe (Reinert et al., 2006). Other studies have shown that both cell-mediatedand humoral responses are not suppressed when mice are treated with this same glutaminase-free asparaginase. Glutamineis important in many cellular processes, notably for providingenergy and nitrogen for the synthesis of DNA and RNA in lymphocytes,and serves to enhance the function of stimulated immune cells.

Furthermore, glutamine is essential for optimal cell functioning of not only lymphocytes but also monocytes and granulocytes (Eliasen et al., 2006). With respect to asparaginase, the depletionof glutamine is suggested to be the primary immune suppressive agent and that the immune suppressive effects of asparaginase are a result of decrease glutamine levels, leading to metabolic stress as evidenced by an increase in the phosphorylation of the translation factor, eukaryotic initiation factor 2 (eIF2)5. They prevent or ameliorate this condition by increasing the supply of glutamine in the diet via unlimited consumption of an alanyl-glutamine dipeptide (AlaGln) solution.

2.3 Chemical features of drug available for therapy:

2.3.1 Native enzyme:

Different isoenzymes of L-asparaginase have been isolated by using different strains of E. coli (Iron &Arens, 1979). The purified E. coliL-asparaginase has a molecular weight of 133–141 kDa . All asparaginases are obviously composed of four subunits with an active site on each subunit and the molecular weight of each subunit is reported to be 22 kDa. Studies of (Korholz et al.,1989) suggest that the molecular weight of each subunit is around 32 kDa for E. coliL-asparaginase and for the Erwinia preparations it happens to be 40 kDa.

2.3.2 Modified enzyme :

High immunogenicity in approximately 25% of the patients to the foreign protein ranging from mild allergic reactions to anaphylactic shocks and very short half-life value restrict subsequent therapy with the nativeL- asparaginase. Attempts have been made to reduce the potential immunogenicity while preserving its activity and prolonging its half-life period, so as to avoid need for frequent intra- muscular injections. Chemical modification to some extent appears to meet these requirements. In this regard, in the mid-1970s, several groups began chemically modifying the L-asparaginase by adopting various methods in an attempt to identify a form that was less immunogenic but retained good antitumor activity **.**

A)PEGylation, the conjugation of L-asparaginase to PEG turned out tobe the most successful method of chemical modification. It was developed in the 1970s and 1980s.

Abuchowski et al.,(1979) were first to successfully couple PEG to L-asparaginase , antileukemic activities of this new preparation were tested in the L5178Y tumor bearing BDF mouse model. Conjugation of enzyme to PEG succeeded in abolishing the drug's immunogenicity. Biochemical properties of PEG-L-asparaginase, commercially known as pegasparagase markedly differ from the native enzyme. Its apparent molecular weight is higher. Its reactivity with specific antibodies is very low.

B)Coupling of L-asparaginase to dextran has also been tried to improve thermal and proteolytic stability, and to reduce immunogenicity but the reduction in the immunogenic toxicity proved to be less effective than with PEG.

C)Uren and Ragin in 1979used poly-dl-alanyl peptides to block immunogenic epitopes of *E. coli* and *Erwinia* L-asparaginase but the clinical studies have not been performed to date.

D)Nerker and Gangadharan in 1989conjugated *Erwinia*,L-asparaginase to human serum albumin.

E)Acylation has also been applied as a method for L-asparaginase modification(Martins et al.,1990) but the limitation of this approach is that enzyme becomes hydrophobic after modification.

L-Asparaginase entrapped in red blood cells was quite stable and had markedly prolonged *in vivo* half-life. (Nagi et al ., 1998).

2.4. Phamakokinetics:

In the treatment of malignant diseases, L-asparaginase is administered by the intramuscular or intravenous route. Most of the research done on the concentration time curves showed plasma half-lives of 4–15 h, some authors suggest that an initial uptake of the enzyme by some tissues is followed by re-distribution into the plasma (Bruck et al .,1989).

Broome,1968 found only low activity in the spleen and serum of mice 24 h after therapy with L-asparaginase, but high hepatic activity, it was proposed that most of the asparaginase is

eliminated via the reticuloendothelial system , Hall (1970)observed rapidly decreasing plasma activity both with E. coli and Erwinia asparaginase, also concluded that the elimination takes place primarily via the reticuloendothelial system. Oettgen et al .,(1970), stated that the higher incidence of severe hypersensitivity reactions after intravenous administration might be attributed to contamination by foreign proteins.

Nesbit et al., (1979) compared the intramuscular and the intravenous route with respect to therapeutic efficacy and untoward effects. They found no significant differences regarding clinical efficacy, but noted anaphylactic reactions in 18 of 87 pediatric patients after intravenous doses while hypersensitivity reactions were only mild in the group of 77 patients who received the drug intramuscularly, The incidence of severe anaphylactic reactions was reported to be the only clinically relevant difference between the two modes of administration, considering the likelihood of contamination of the asparaginase preparations by endotoxins and other bacterial proteins, especially in the early years of clinical therapy.

Since the dosage and therapy schedule required for optimal clinical efficacy were not known at the time when the asparaginases were introduced into clinical use , the enzyme was employed at a variety of different dosages and schedules ,These ranged from 10 U/kg BW daily to repeated single administration of 25000 U/m2 BSA and 20000 U/kg BW (Fallet et al .,1985) . As the long-term outcome of the disease was the only parameter available to establish the required dosage, the usual approach was to choose fairly high doses in order to avoid treatment failures from, underdosage, which might be incorrigible later on. While a clear relationship between the dose and the degree of toxicity was not established, a higher incidence of unfavourable side effects was observed with high doses and sometimes attributed also to preparations containing E.coli asparaginase in comparaison to the Erwinia enzyme.

Edema and necrosis of pancreatic islets were observed in rabbits following a single, intravenous injection of 12, 500 to 50, 000 International Units Elspar/kg (approximately equivalent to 25 to 100-fold the recommended human dose, when adjusted for total body surface area). These changes were not reflective of pancreatitis, and were not observed in rabbits following a single intravenous injection of 1000 International Units/kg (approximately equivalent to two times the recommended human dose, when adjusted for total body.

Elspar was evaluated in an open-label, multi-center, single-arm study in which 823 patients less than 16 years of age with previously untreated acute lymphoblastic or acute undifferentiated leukemia received Elspar as a component of multi-agent chemotherapy for induction of first remission. Elspar was administered at a dose of 6, 000 International Units/m²intramuscularly 3 times a week for a total of 9 doses.Of 815 evaluable patients, 758 (93%) achieved a complete remission. In a previous study, in a similar patient popula-tion, which utilized an initial induction chemotherapy regimen containing the same agents without Elspar, 429 of 499 (86%) patients achieved a complete remission.

2.5 Drug interactions:

Clinically relevant interactions between asparaginase and other drugs employed in the treatment of malignant diseases are based on the enzyme-induced impairment of the protein synthesis, and the synthesis of DNA and RNA .The prior or concurrent administration of agents that inhibit protein synthesis and of antimetabolites or alkylating agents has been shown to impair the cytotoxicity of these enzymes considerably. (Martin, 1969) .

Those findings prompted Capizzi and Cheng 1981to perform further studies on cell cultures, in animal experiments and in a small number of patients. He found that both the sequence and the time interval between the dose of L-asparaginase and the antimetabolite,[methotrexate (MTX)] , affects the therapeutic outcome. A total of 55% of mice that had leukaemia cell transplants and received several treatment cycles of MTX followed by L- asparaginase were cured, while no response was noted with the opposite sequence of administration, research done on the optimal time interval between the MTX and the L-asparaginase administration yielded an interval of 9–10 days to be appropriate when asparaginase was given prior to MTX, as compared to 24 h between the drugs when the opposite sequence was used (Lobel et al ,1979).

L-asparaginase should not be used by vein with or immediately before vincristine or prednisone because very serious side effects such as blood problems (erythropoiesis) or numbness, tingling, burning, or pain in the hands or feet may occur.drugs affected
by liver enzymes (such as cyclophosphamide,methotrexate,vincristine).

L-sparaginase can either decrease the beneficial effect of these drugs or increase their side effects when given before or at the same time as these drugs.

2.6 Clinical efficacy:

The observed in vitro antitumour activity of different asparaginase preparations prompted their utilisation in the treatment of various different tumour entities. Studies showed that apart from few exceptions , satisfactory or good clinical efficacy could only be expected in the treatment of haematological diseases. Acute lymphoblastic leukaemia and some non-Hodgkin lymphomas of childhood in particular responded in such a favourable manner that soon L-asparaginase was firmly established as a component of the induction protocols for the treatment of these diseases.The age of patients treated with L-asparaginase ranges from infancy to adults in advanced age.

L-asparaginase for induction or reinduction is usually combined with prednisone and vincristine, but successful monotherapy has also been reported. Jaffe et al.,(1971) observed complete remission in 20% of patients treated for persistent or relapsed acute lymphatic or acute myelogenous leukaemia, while a different study reported dose-dependent remission induction in 10–63% of patients.

L-asparaginase has become an integral part of the modern day chemotherapy protocols. It has been shown to improve event free survival when used during the intensification/consolidation phases of ALL treatment procedure (Clavel et al .,1980) particularly for patients with high-risk features at diagnosis including T-cell phenotype or early slow response to standard induction chemotherapy.

Clavel et al. used a four-agent induction regimen (prednisone, vincristine, methotrexate and doxorubicine) followed by multiple agent therapy including weekly *E. coli* L-asparaginase at 25,000 U/m2 i.m. (intramuscular). The results were excellent and have hardly been improved in subsequent studies. In 1987, Pediatric Oncology Group (POG) conducted a randomized trial (POG8704) designed to evaluate the efficacy of high L-asparaginase consolidation (25,000 IU a week for 20 weeks) as a part of multiagent chemotherapy regimen in patients newly diagnosed

with T-lineage ALL or advanced-stage lymphoblastic lymphoma. The results reaffirmed the clinical importance of L-asparaginase, as patients treated with the L-asparaginase containing regimens achieved improved disease-free survival as compared with patients treated without L-asparaginase (Amylon et al., 1999).

Pegaspargase can be used in reinduction and maintenance therapy of patients with ALL who have relapsed while receiving chemotherapeutic regimens that have included native L-asparaginase preparations. L-Asparaginase has also been found to be effective against meningeal leukemia.(Bushara and Rust , 1997).

2.7 Resistance to the drug:

Earlier in the development of L-asparaginase as an antineoplastic agent, it was speculated to kill leukemic cell selectively without affecting normal cells. This turned out to be a simplistic paradigm as resistance to the drug emerged, primarily via de-repression of the asparagine synthetase gene in tumor cells. sparagine synthetase is the enzyme responsible for the synthesis of L-asparagine in normal cells. Andrulis and Barrett 1989, and Greco et al. in the 1989attributed the regulation of the expression of asparagine synthetasegene to the degree of methylation of cytosine residues in DNA (Greco et al.,1989).

Pre-clinical and clinical synergies between L-asparaginase and cytosine arabinoside have been ascribed to lowered activity of L-asparagine synthetase secondary to increased methylation of cytosine residues in the gene encoding this enzyme (Nyce, 1989). Accelerated clearance of L-asparaginase following induction of specific antibodies has also been found as a potential reason for resistance (Capizzi, 1993).

The view that antibodies have a role to play in drug resistance was also supported by Cheung et al., 1986), who studied 13 patients under going conventional chemotherapy for ALL and found six patients with low anti-L-asparaginase antibody titers remained in continuous complete remission while four of the seven patients with significant titer had relapsed. Asselin et al. , (1993) and (Gentili et al., 1996) demonstrated normal plasma levels of L-asparagine shortly after drug administration in patients who had exhibited hypersensitivity to *E. coli* and *Erwinia*L-asparaginases respectively.Investigation of Killander et al., 1976)revealed that despite immunization of patients, the drugs efficacy remained un impaired. While the development of

antibodies does seem to find correlation with diminished drug effect, the clinical significance of such antibodies has been questioned.They speculated on another mechanism: the pool of L-asparaginase sensitive cells may produce cytokines that control the expansion of resistant cells. As soon as sensitive cells are killed by L-asparaginase, resistant cells escape from regulatory control.

Holleman et al.,(2003)have associated the resistance to different classes of drugs with impaired apoptosis in childhood ALL.The authors described caspase-3 or PARP[poly(ADP-ribose) polymerase] inactivation to be responsiblefor resistance to L-asparaginase and prednisolone, another chemotherapy agent used in multi-agent regimen protocol.

2.8 Toxicity of L-sparaginases:

2.8.1 Immunological reactions:

L-sparaginases are associated with a unique set of side effects. Hypersensitivity reactions, due to anti asparaginase antibody production, have been observed in up to 60% of patients at some time during E. coli asparaginase therapy. The development of these antibodies appears to be more commonly observed with native E. coli asparaginase .compared with the pegylated enzyme (Avramis et al.,2002,Hawkins et al.,2004) Symptoms of clinical hypersensitivity include

anaphylaxis, pain, edema, urticaria, erythema, rash, and pruritis (Woo et al.,2000). The route of administration determines the clinical symptoms with a greater incidence of major skin reactions observed with intramuscular (i.m) administration compared with intravenous(iv) administration.

Combination chemotherapy approach rather than monotherapy is considered helpful in avoiding allergic reactions, because the former produces immunosuppression, which obliterates an immune response to L-asparaginase.

Clinical hypersensitivity occurs almost exclusively in post induction regimens (i.e, intensification, reinduction) when asparaginase has not been given for weeks or months but there are several possible explanations for the rarity of allergic reactions during remission induction. For example, there is a delay in an effective immune response due to the time taken for complement activation and the subsequent production of antibodies, the symptoms associated with allergy might be masked by intensive corticosteroids treatment that occurs during induction, and the frequency of dosing during induction may have a desensitizing effect, as allergic reactions are rarely observed in this phase despite measurable antibody production.

Some studies have shown that the incidence of hypersensitivity to L- asparaginase is similar between age groups(Silverman et al.,2001;Barry et al.,2007), although others have suggested that infants and younger patients develop antibody and hypersensitivity reactions less frequently than teenagers and adult patients (Avramis andTiwari,2006).

2.8.2 -General Tissues Toxicity:

Since the mechanism of action of L-asparaginase is fundamentally different from other agents employed in the treatment of malignant diseases, only low toxicity toward normal cells and organ systems was assumed when the enzyme was first introduced into treatment protocols.

When larger numbers of patients had been treated it was apparent however, that, apart from immunological reactions toward the foreign macromolecule, healthy tissue was also frequently affected. Those effects have in the main been attributed to an impaired protein synthesis. The impairment of protein synthesis affects all tissues relative to their synthetic capacity, and the

asparaginase-induced untoward effects thus involve many organ systems.The number of patients affected to a clinically relevant degree is small and has decreased with use of the better purified preparations currently available.

2.8.3- Liver toxicity:

The hepatotoxic effects of E.coli asparaginase are well documented in both human and animal systems (Canellos et al., 1969). It has been suggested that the toxic side effects of asparaginases having glutaminase activity may reside in the capability of these enzymes to deplete both asparagine and glutamine. The biosynthesis of asparagine in mammalian systems is primarily mediated through a glutamine-dependent transamidation reaction catalyzed by asparagine synthetase (Woods and Handschumacher,1971). Therefore, glutamine deprivation may block the biosynthetic pathway by which normal cells escape the toxic effects of asparagine depletion. Studies demonstrating that the mammalian liver is the organ primarily responsible for the homeostatic regulation of asparagine suggest an important physiological role for asparagine synthetase in this organ.

Asparagine synthetase is an inducible enzyme in ratliver, and itsactivity increases during regeneration, nutritional deprivation of asparagine or asparaginase therapy Patterson and Orr,(1969). The inducible nature of asparagine synthetase may explain why E. *coli asparaginase treatment is capable of inhibiting only the early* wave of mitosis in regenerating rat liver after hepatectomy and not subsequent waves of mitosis (Becker and Broome, 1967). Moreover, enzyme induction may be important in alleviating asparaginase-induced hepatotoxicity as suggested by (Durden et al ., 1983) that liver lipid concentrations and plasma levels of lipid and proteins synthesized in the liver return to normal by the third week of treatment. Also , the authors indicate that treatment of mice for extended periods of time witha glutaminase-free asparaginase from v. succinogenes is not hepatotoxic.

Their kinetic analysis of E. coli asparaginase-induced hepatotoxicity revealed that marked toxicity was evident during the first 2 weeks of treatment followed by a recovery to normalcy. Liver lipid levels increased rapidly during the first 2 weeks correlating with decreased plasma levels of albumin, antithrombin III, cholesterol, and triglycerides during the second and third

weeks of E.coli asparaginase treatment, both animal weight and liver weight were significantly reduced as compared to controls.
The most significant absolute increase in total extractable hepatic lipid occurred during the first week of E. coli asparaginase treatment followed by a resumption to normal levels. In patients treated with E.coli asparaginase for prolonged periods, a similar pattern of recovery is observed (Canellos et al .,1969).

While increases in the parameters of hepatic functions are frequent under asparaginase therapy, those changes rarely reach clinical significance.

Oettgen et al. 1970 , in their study of 131 children and 143 adults, found a dose-independent increase of alkaline phosphatase in 31% of the children and 47% of the adults, and a rise inaspartate aminotransferasein 46% of the children and 63% of the adults. Other relevant increases concerned the serum levels of bilirubin and 5%-nucleotidase, and there was also an enhanced retention of bromsulphthaleine.

Changes of the hepatic structure due to fatty degeneration have been diagnosed at autopsy in 40 of 55 patients. In those cases, a relationship between the last asparaginase dose and the degree of fatty degeneration was likely. Pratt and Johnson (1971) , doing an autopsy study cautioned that hepatic lipoidosis may persist for up to 261 days after the last dose of L-asparaginase in children who died from various hematologic malignancies. There was a suggested relationship between the severity of the hepatic changes and dose. Recent data suggest that a greater number of adult patients with ALL have hepatotoxicity reactions in response to asparaginase therapy compared with pediatric populations (elevated liver enzymes, 36% vs 20%, hyperbilirubinemia, 14% vs 3%; hypofibrinogenemia, 16% vs 2%)(Earl, 2009). Advani et al 2007showed that IV pegaspargase is hepatotoxic in approximately one third of adult patients and they reported elevated liver enzymes in 51% of adult patients treated with IV pegasparaginase, and that pegasparaginase.-associated hepatotoxicty reactions have generally been reported to be mild and transient (Rytting et al., 2008).

2.8.4 Coagulation system:

As a result of impairment of protein synthesis, changes in the coagulation function which manifest themselves as haemorrhage, disseminated ` or thrombotic events, may be of considerable clinical relevance. Often, in these patients, who are seriously affected by their disease and other medication which acts on the bone-marrow, the occurrence of haemorrhage cannot be related unequivocally to the asparaginase therapy.

In 1970, Oettgen et al. reported bleeding events in 6% of L-asparaginase-treated patients, but the great majority of patients had terminal stage disease with severe thrombocytopenia and sometimes bleeding from their tumour. In another study on 238 adult and pediatric patients bleeding occurred at a rate of 2.1%, with fatal outcome in one case, and 4.2% of treated patients had thromboembolic complications.

The adverse events related to coagulation disorders are a product of the drugs effect on protein synthesis .The most consistent findings are reductions in plasminogen, fibrinogen, antithrombin and factors IX and X with prolongation of activated partial thromboplastin time.Venous thrombosis is one of the more common serious complications of the treatment of childhood acute lymphoblastic leukaemia (ALL). A meta-analysis of 1752 patients from 17 prospective trials demonstrated a rate of symptomatic thrombosis of 52% (Caruso et al., 2006).

Eetiology is multi-factorial and is probably related to increased thrombin generation at diagnosis, as well the pro-coagulant effects of high-dose corticosteroids and profound Asparagine depletion and prolonged deficiency of anti thrombin III and other natural anticoagulants particularly during induction. This risk is compounded by the presence of central venous lines (CVL) and, possibly, hereditary thrombophilia . There is now strong evidence that optimal use of L-sparaginase can improve outcome in ALL. Thus, clinicians managing children with ALL and a therapy associated thrombosis are faced with a significant dilemma: should they re-expose the patient to L-sparaginase with the attendant risk of recurrent thrombosis or omit Asparaginase from therapy and thereby increase the risk of relapse Payne & Vora, (2007). report the incidence and outcome of thrombosis in the prospective multicentre trial UK ALL 2003. Suggested that re-exposure of those children who have suffered a venous thrombosis in association with L-sparaginase, is safe particularly if undertaken with heparin prophylaxis.

2.8.5 Gastrointestinal system:

More than half of all patients develop mild to moderate loss of appetite, nausea, vomiting, and a mild increase of body temperature (Pratt et al.,1971).Moreover, Oettgen et al.,(1970) in a study on 131 children and 143 adults found a relevant decrease in bodyweight in 61% of the pediatric and 76% of the adult patients; they also pointed out that there might be hypoproteinaemia related fluid retention and that a bodyweight decrease beyond the measured values consequently should not ruled out.

2.8.6Bone marrow function:

In addition to the effects on the coagulation system, the asparaginase treatment is also associated with direct effects on bone marrow function in the form of only mild to moderate suppression of all three cell lines (Johnston et al.,1974). The enzyme-induced functional impairment, however, rarely reaches clinical significance and therapeutic consequences are rarely required .

2.8.7 Lipid metabolism:

Changes in lipid metabolism in the form of hypo- or hyperlipidaemia are frequently encountered with asparaginase treatment . One cannot rule out that these changes might be related to the concurrent administration of glucocorticoids. Oettgen et al.,(1970) reported a decrease in serum cholesterol in 80% of patients studied, other authors have found sometimes extreme reversible increases of the triglyceride level under asparaginase (Hoogerbrugge et al.,1997). The mechanismof L-asparaginase–associated hypertriglyceredemia is related to an increase of the VLDL fraction (very low density lipoprotein), and an decreased lipoprotein lipase activity which is a key enzyme in the removal of triglycerides from plasma , and /or exogenous chylomicrons.Several reports confirm that administration of L-asparaginase as monotherapy or in combination with prednisone, is associated with lipid abnormalities in children receiving treatment for ALL. , but there are no data available concerning this toxicity in adults.

The changes in serum cholesterol levels are consistent with the known association between hypercholesterolemia and corticosteroids . It is well established that corticosteroids alter lipid and lipoprotein metabolism by increasing hepatic cholesterol synthesis (Cremer et al.,1988).

These lipid abnormalities during remission induction chemotherapy for ALL have been reported to be benign and transient. Due to its transient character, no changes in the treatment of the

underlying disease are recommended. Few cases are reported with severe symptomatic hyperlipidemia requiring therapeutic interventions.

2.8.8 Serum Proteins:

In many patients, the impairment of protein synthesis induces a decrease in serum proteins. (Oettgen et al.,1970) found a reduction of serum albumin in about 80% of patients independent of the L-asparaginase dose used. The reduction in the fractions of albumin, ά2, and ßglobulins was significant, while the α1 fraction was not significantly different from baseline values. As the serum albumin is of particular clinical impact due to its drug binding and transport function, potential changes in the serum level should be appropriately considered in all treatment options.

2.8.9 Central nervous system:

Neurotoxicity (depression, lethargy, fatigue, somnolence, confusion, irritability, agitation, dizziness) occurs in up to25% of adult patients treated with l-asparaginase, but rarely occur in children. Neurotoxity may also result from lack of L-asparagine and L-glutamine in the brain.Ohnuma et al., (1969) reported three patients with severe central nervous disorders who had a marked improvement of symptoms after administration of asparagines. Another study reproducibly established a relationship between marked neurological symptoms and a pronounced increase of the ammonia blood level (Leonard and Key 1986), but this was not confirmed by others. There have also been reports on EEG changes such as a decrease in alpha wave activity and an increase in theta and delta activity.

2.8.10 Pancreatitis:

L-Asparaginase-associated pancreatitis (AAP) is defined as acute pancreatitis in patients that are receiving L-asparaginase treatment at the time of onset of acute pancreatitis.

The risk of pancreatitis, although low, seemed well established for some of these agents.

Azathiaprine	Oral contraceptives
L-Asparaginase	Pentamidine
Chlorthalidone	Procainamide
Ethanol	Rifampicin
Furosemide (Lasix)	Sulindac
6-Mercaptopurine	Tetracycline
Methyldopa	Thiazide diuretics
	Valproic acid

Table. (2. 1):Agents and Drugs Reported to Cause Pancreatitis in Humans (Longnecker & Wilson,1991).

The origin, formulation, dosage or method of administration of asparaginase does not seem to influence the risk of AAP (Alvarez &Zimmerman,2000; Knoderer et al., 2007; Kearney et al., 2009).

Pancreatitis is defined as the histological presence of inflammation within the pancreatic parenchyma. Acute pancreatitis is a reversible process characterized by the presence of interstitial oedema, infiltration by acute inflammatory cells, and varying degrees of apoptosis, necrosis, and haemorrhage.

This pancreatic inflammation probably reflects premature activation of intraacinar pancreatic proenzymes or zymogens within the acinar cells (Gorelick &Thrower, 2009). The activated zymogens, especially theprotease trypsin, cause injury to the pancreatic acinar cells, which leads to the production and release of a cascade of cytokines (Pandol, 2006) and an inflammatory response, which may include systemic inflammatory response syndrome and multiorgan failure (Gardner et al.,2009; Malmstrom et al., 2012). The pathophysiology behind AAP is unknown Vrooman et al.,(2010), but is regarded to reflect systemic depletion of asparagine with a subsequent reduction of protein synthesis, especially in organs with high protein turnover, such as the liver and pancreas.

Genetic predispositions are likely to play a role, because AAP occurs after one or a few administrations of L-asparaginase with a high likelihood of recurrence at re-exposure, although the absolute risk has not been determined (Flores-Calderon et al.,(2009).

Although not validated in the pediatric population ,the Atlanta criteria for acute pancreatitis require the presence of at least two of the following three criteria : clinical presentation resembling pancreatitis, amylase or lipase more than three times the upper normal level (UNL), and imaging compatible with acute pancreatitis .

Alternatively, acute pancreatitis can be classified according to the Common Terminology Criteria for Adverse Events that, as opposed to the Atlanta criteria, grade the severity of the pancreatitis (grade 1–5). Until a consensus on the classification of AAP is reached among pediatric oncologists, (Raja et al., 2012) recommended that the Atlanta criteria are applied, as this is the grading system most commonly used by those involved in the diagnosis, monitoring and treatment of pancreatitis in general, i.e. gastroenterologists, pathologists, radiologists, and surgeons. the Atlanta criteria identify acute pancreatitis as either non-severe or severe, where a duration of more or less than 48 h discriminates between the two (Zaheer et al., 2012).
The clinical presentation, imaging methods, biochemical markers and complications in AAP do not differ significantly from acute pancreatitis in other pediatric populations.Still, the course of pancreatitis in leukaemia and lymphoma patients treated with L-asparaginase could be influenced by their immune suppression, frequent microbial translocation from the gut, coagulation disturbances and hyperlipidaemia associated with asparaginase-containing combination chemotherapy. Studies assessing AAP retrospectively report incidences of AAP between 6.7% and 18% ,the differences in incidence primarily reflects different definitions of AAP. Thus, mild AAP included in the study by Knoderer et al(2007) would not be considered to be AAP in any of the other studies. Among studies with a high degree of consensus on pancreatitis definitions, the incidence of AAP is 5–10%.

2.8.10.1Clinical presentation:

Patients with AAP typically present with nausea, vomiting, and sudden abdominal pain, which is most commonly located in the epigastric region. Patients may also present with pain radiating to the back or shoulders . Other symptoms include low grade fever, and pleural effusion Top et al., (2005); Raetz & Salzer, 2010; Stock et al, 2011). Upon examination, patients tend to lie still, because movement aggravates pain. The child may be irritable, quiet, or both. Decreased bowel sounds and rebound abdominal tenderness can be seen in severe cases, Werlin et al., (2003).

If AAP is complicated by a systematic inflammatory response syndrome, then tachycardia, hypotension and fever will also be present, and the clinical presentation may easily be misinterpreted as septicaemia (Bradley, 1993). Elevations in serum amylase and lipase are the most common biochemical characteristics of pancreatitis. Patients with acute pancreatitis can present with normal amylase and elevated lipase levels and vice versa, thus highlighting the importance of using both biomarkers.

2.8.10.2Imaging:

Imaging includes ultrasonography and computerized tomography(CT).

Ultrasound may be normal in mild cases, but can reveal increased pancreatic size and decreased echogenecity in severe cases. Although the avoidance of irradiation and the easy bed-side applicability makes ultrasonography attractive when AAP is suspected, it is burdened by its operator- dependent sensitivity and is furthermore difficult in cases of obesity or overlying bowel gas (Baillie, 2007). Contrast enhanced CT is useful for the detection of pancreatic necrosis, if performed days or even weeks after the initial presentation of AAP. There are no current guidelines recommending the use of magnetic resonance imaging (MRI).

Adult studies indicate that MRI provides much the same information as CT, but experience is limited in children (Tipnis et al, 2008).

2.8.10.3Treatment:

Treatment of acute pancreatitis is primarily supportive and aims to reduce symptoms and monitor potential complications . Petrov et al., 2006; Wu et al., 2010). Several randomized clinical trials of non-asparaginase related pancreatitis in adults have shown that early enteral feeding reduces the incidence of complications.

The early administration of adequate fluid resuscitation is generally recommended. More applied treatments in case of severe pancreatitis are administration of the synthetic somatostatin analogue Octreotide or continuous regional arterial infusion of protease inhibitors and antibiotics (Morimoto et al., 2008; Wu et al, 2008).

Somatostatin (Octreotide) inhibits secretions from a wide range of endocrine organs, including the pancreatic digestive enzymes, and may thus decrease the pancreatic inflammation. Administration of Octreotide to rats receiving L-asparaginase has been effective in preventing injury to the pancreatic acinar cells. It has been demonstrated to be safe and effective in adults with acute pancreatitis , but, beyond a few case reports, little is known with respect to AAP in children. Octreotide was administered to four out of five paediatric patients with AAP.

Although all four patients subsequently demonstrated clinical and laboratory improvements two of the patients subsequently developed persistent insulin-dependent diabetes mellitus. There are no large studies of Octreotide treatment in children with AAP, or other children with acute pancreatitis.

In addition, there is no consensus on when to treat patients, which doses to use, how long to treat, and the pattern of side effects. In the case reports patients were treated with doses that ranged from 2.5 to 7.2 lg/kg per day (Suzuki et al., 2008;Hatzipantelis et al, 2011; Tokimasa & Yamato, 2011). Continuous regional arterial infusion of protease inhibitors has been used in patients with necrotizing pancreatitis.

Concomitant with the protease inhibitors, antibiotics are administered intravenously(Piascik et al., 2010). A large adult trial showed continuous regional arterial infusion of protease inhibitors and antibiotics to be effective in preventing complications and in reducing mortality rates in severe acute pancreatitis.

Pediatric experience remains limited. One study included five pediatric patients with severe AAP that were treated with continuous regional arterial infusion within 48 h of diagnosis. All five patients had favourable clinical outcomes and could resume chemotherapy within 22 d, although none received further L-asparaginase therapy (Morimoto et al., 2008).

2.8.10.4Complications:

Acute severe complications to acute pancreatitis include systemic inflammatory response syndrome and multiorgan failure, with the lungs and kidneys most commonly affected. Patients may develop pleural effusions, toxic pneumonia, acute respiratory distress syndrome, and renal

failure (Bassi et al.,2003; Pastor etal.,2003). Short term complications of acute pancreatitis include pancreatic necrosis and the formation of pseudocysts (Bai et al, 2011). In addition, the necrotic pancreatic lesions can become infected. Such necrosis will typically occur after the first week (Zavyalov et al., 2010). The role of antibiotics in pancreatitis in general has changed. It was previously believed that all patients with pancreatic necrosis should receive antibiotics , but it is now recommended not to administer antibiotics unless infected necrosis has been confirmed clinically or by fine needle aspiration. If such patients continue to be burdened by severe pain, surgical removal of necrotic areas may be indicated (Pattillo & Funke, 2012). However, these data stem from immunocompetent patients with acute pancreatitis .

(Dellinger et al., 2007; Garcia-Barrasa et al, 2010), and need to be modified in immunosuppressed cancer patients receiving L-asparaginase. Since AAP at presentation may be diffcult to distinguish from septicaemia, broad-spectrum antibiotics should be administered until infection has been ruled out. In the presence of necrosis, with or without infection, future L-asparaginase therapy should be permanently discontinued .

Pseudocysts can emerge as a complication to AAP. Such cysts contain pancreatic juice enclosed by a non-epithelialized wall (Banks & Freeman, 2006; Zaheer et al, 2012). Although, most pseudocysts have been reported to occur within 4 weeks of acute pancreatitis,there are no large series that describe this in detail for patients with AAP. Generally, pseudocysts should be managed conservatively, as most cases resolve during the subsequent weeks or months, and the likelihood hereof does not seem to be associated with cyst size. Intervention is indicated in patients that have persistent symptoms, such as severe pain, despite supportive care, or in case of infection or bleeding (Gumaste & Aron, 2010).

Persistent pain can be caused by pressure on surrounding structures from the pseudocyst, infections or persistent inflammation. Habashi & Draganov, 2009). When indicated clinically, endoscopic ultrasoundguided percutaneous drainage of pseudocysts is feasible and safe even in children (Jazrawi et al., 2011). In the presence of pseudocysts, L-asparaginase treatment should be permanently discontinued. . Changes in the endocrine and exocrine pancreatic function, predominantly in the form of an impaired glucose metabolism, are frequently observed under asparaginase treatment. Severe diabetic ketoacidoses as well as non-ketotic, hyperosmolar

hyperglycaemias, which usually respond to exogenous insulin, have been observed. While these findings are usually attributed to an impaired insulin availability due to the effects on the protein synthesis. Some authors propose an impairment of insulin secretion and a reduction in insulin receptors.

Furthermore, an effect on both alpha and beta cells and an ensuing insulin deficiency and concurrent hyperglucagonaemia is assumed which translates into an impairment of glucose tolerance. The probability of hyperglycaemic states is related to age, obesity and the diagnosis of Down's syndrome, with children older than 10 years carrying a markedly increased risk. Long term consequences of AAP include chronic.

Re-introduction of L-asparaginase:

Three studies have described the re-administration of L-asparaginase after the occurrence of AAP. The rate of AAP when L-asparaginase was re-introduced was reported to be 0% (0 out of one patient, 7.7% (two out of 26 patients), and 62.5% (10 out 16 patients). The difference in incidence of AAP after reintroduction of L-asparaginase in the two larger studies primarily reflects the criteria for reintroduction, being mild AAP and complete resolution of symptoms in one study (Knoderer et al, 2007), whereas the other study only required resolution of symptoms within 72. Reduced L-asparaginase exposure due to discontinuation of therapy as a result of toxicity has been related to decreased event-free survival in childhood ALL (Silverman *et al*., 2001); it is therefore important that ALL protocols include recommendations regarding the re-introduction of L-asparaginase treatment after AAP.Based on the Atlanta criteria, (Raja *et al*., 2012) recommended thatL-asparaginase is re-introduced to patients who, within 48 h, have.

i) no AAP symptom

ii) amylase and lipase levels below three times the UNL,

iii) no pseudocysts or necrosis on imaging.

If such patients experience a new episode of AAP, L-asparaginase therapy should be permanently terminated .

Possible risk factors for developing AAP:

Higher age, concomitant treatment with other anti-cancer drugs (e.g. 6-mercaptopurine, glucocorticoids and daunorubicin), certain genetic polymorphisms, and severe hypertriglyceridaemia have all been indicated to increase the risk of developing AAP. However, once L-asparaginase has been permanently discontinued, the patients no longer experience acute pancreatitis. A genetic predisposition could involve multiple pathways, such as the propensity for activation of proenzymes and degradation of enzymes, extracellular matrix integrity and the inflammatory profile (Andersen et al, 2005).

Although nucleotide sequence variants in several genes (e.g. CFTR, CTRC, PRSS1 and PRSS2) have been linked to the risk of pancreatitis in general (Whitcomb, 2010), no specific genetic polymorphisms have been associated with AAP.

The cystic fibrosis transmembrane conductance regulator gene (CFTR) is involved in eliminating trypsin from the pancreas through flushing of trypsin into the duodenum, thus removing it from the pancreatic duct. The CFTR Ile556Val and 470Val polymorphisms have been shown to be associated with pancreatitis in patients with hyperlipidaemia (Chang *et al*, 2008). The chymotrypsin C gene (CTRC) codes for the protein Chymotrypsin C, which plays a role in the breakdown of prematurely activated trypsin (Whitcomb, 2010). Variations in the CTRC gene have been associated with a fivefold increased risk of developing chronic pancreatitis. The protease, serine, 1 and 2 genes (PRSS1 and PRSS2) are involved in the synthesis of trypsinogen . A deficiency in the trypsin protein can increase the risk of pancreatitis, either because of premature activation within the pancreas, or due to lack of inactivation of the enzyme. Mutations in the PRSS1 gene are demonstrated in patients with hereditary pancreatitis. Gain-of-function mutations are suspected to be responsible for many cases of hereditary pancreatitis in Caucasians.

Transient hypertriglyceridaemia is a frequent complication to L-asparaginase therapy (Cohen *et al*., 2010). In patients not receiving L-asparaginase, the risk of acute pancreatitis is increased when severe hypertriglyceridaemia (i.e. levels above 11.3 mmol/l) is present (Yadav & Pitchumoni, 2003).Hypertriglyceridaemia is frequently encountered in patients treatedwith L-asparaginase, especially when given in combination with steroids Salvador *et al*, (2012) Hypertriglyceridaemia is transient in patients receiving L-asparaginase.

In the presence of hypertriglyceridaemia, the triglycerides may be hydrolysed within the pancreas by the pancreatic enzyme lipase. The fatty acids that are thus released may in turn cause acinar cell injury, activation of trypsinogen and initiation of AAP. Notably, the measured serum levels of amylase and lipase may be falsely low or even normal in patients with the combination of pancreatitis and hypertriglyceridaemia because plasma lipids can interfere with the assays .There is no consensus on when and how to treat hypertriglyceridaemia, if it occurs during L-asparaginase therapy, or even if such lipid-reducing therapy influences the risk of AAP.

Applicable treatment modalities include dietary restrictions, fibrates, statins, insulin-glucose infusions, heparin infusions, and plasmapheresis. Generally, triglyceride levels are lowered significantly in a few days, but the efficacy of these approaches have not been formally evaluated (Dietel et al, 2007; Ridola et al, 2008; Solano-Paez et al., 2011). For adolescent and adult patient with asparaginase-associated hypertriglyceridaemia above 11.3 mmol/l, but without pancreatitis, it is recommended that such patients should be followed closely for pancreatitis, and that L-asparaginase therapy should be withheld until triglyceride levels have normaliz (Stocket al,2011).However, such expert panel reommendations are not available for children.

Due to the suspected link between hypertriglyceridaemia and AAP (Raja et al 2012) recommended :

(i) that patients with AAP have their triglyceride levels monitored,

(ii) that lipid-lowering strategies are applied when triglyceride levels are above 11.3 mmol/l in such patients

(iii) that L-asparaginase treatment is withheld, in children with hypertriglyceridaemia without AAP, until triglyceride levels are below 11.3 mmol/l.

If triglyceride levels do not resolve within a few days on a low-fat diet, treatment with fibrates, statins, insulin- glucose infusions, heparin infusions and/or plasmapheresis are options.

Experimental Animal Models of Acute Pancreatitis:

There are 2 principal functions for animal model research in acute pancreatitis. These are investigations of the molecular mechanisms underlying the pathobiologic responses and testing of potential therapies before human trials, at present, only animal models provide the ability to

reveal the sequence of initiating molecular steps resulting in the pathobiologic processes of acute pancreatitis (Pandol et al , 2007).

Moreover, there are considerable difficulties in designing human clinical trials related to the fact that the disease varies widely in course and severity. In addition, there is a low incidence of the most severe forms of pancreatitis in which testing agents for therapeutic benefit would have the most value. Animal models of acute pancreatitis can be used to screen potential therapies so that only the most promising ones advance to human testing.

There have been several animal models of acute pancreatitis developed(Foitzik et al.,2000;Gukovaskaya et al.,1996). The more commonly used models are listed in (Table .2.2).

For testing a potential therapy, animal models with more severe pancreatitis and systemic inflammatory response should be used because this is the type of disease in which therapy will likely have the greatest benefit in humans.

(Table.2.2). Models of Experimental Acute Pancreatitis

Models Species Features(Foitzik et al,2000;Gukovaskaya et al,1996).

Cholecystokinin analogues (parenteral)	Rat	Pancreaticinflammation, apoptosis,mild necrosis,systemic inflammation
Cholecystokinin analogues (parenteral	Mouse	Pancreatic inflammation,sever necrosis.Systemic inflamation
Pancreatic duct obstruction	Rat	Pancreatic apoptosis
Pancreatic duct obstruction	Opossum	Pancreaticinflammation,Sever necrosis.Systemic inflamation
Bile acid perfusion of pancreatic duct	Rat	Pancreatic inflammation

2.8.10.5Pathophysiology:

Acute pancreatitis can belimited to a local inflammatory disease of the pancreas, or can evolve to severe acute pancreatitis with systemic complications. Three different phases can be distinguished in the development of acute pancreatitis: the first two take place in the pancreas itself, while in the third and final phase extrapancreatic symptoms may occur. However, not all patients will progress to the second and third phase, and in patients who develop mild disease, the disease will not spread outside the pancreas. The first phase takes place in the acinar cell of the pancreas itself ; the acinar cell is damaged and this leads to cell death; this initiates the second phase of local inflammation ofthe pancreas which causes the typical local signs and symptoms.

These first two phases occur to some extent in all patients, but in a number of them, this local process activates the SIRS which makes up the third and final phase. This SIRS response leads to distant organ damage.

Phase 1: Cellular Damage:

Premature conversion of trypsinogen into trypsin, a proteolytic enzyme responsible for activation of a number of digestive enzymes in the gastrointestinal lumen, is considered the starting event in the development of acute pancreatitis. (Bhatia et al., 2005) . Premature activation may occur anywhere along the pathway between the site of digestive proenzyme (zymogen) synthesis within the acinar cell to the ampula of Vater where the zymogens normally enter the duodenum**.** This activation of trypsinogen is normally mediated by *enterokinases*, an intestinal brush border enzyme,cleaves a small trypsinogen activation peptide from trypsinogen to generate trypsin . Trypsin also activates trypsinogen, and trypsin efficiently activates all other pancreatic digestive enzymes. This process occurs in the intestinal lumen . (Whitcomb and Cohn, 2006)Trypsin activity is controlled within the pancreatic acinar cell by being synthesized in the inactive form as trypsinogen. Human trypsinogen can slowly autoactivate to trypsin, which can initiate the zymogen activation cascade. This is prevented by three mechanisms:

The first is packaging of zymogens within dense granules in a semicrystallized form that is separated from some of the lysozomal enzymes cathepsin B- a proteolytic enzyme, under normal conditions responsible for degrading unneeded cellular material and stored in a separate compartment – with trypsinogen in the same intracellular vacuoles . Saluja et al., (1997) in their study inhibited the cathepsin B in vitro using the highly specific, cell-permeant inhibitor E64D,and thus preventing the supramaximal secretagogue-induced activation of trypsinogen.

The second is synthesis of pancreatic secretory trypsin inhibitor (PSTI), also known as serine protease inhibitor Kazal type1(SPINK1). This specific trypsin inhibitor is regulated as an acute phase protein and the ratio of SPINK1 to trypsin is therefore dependent on the status of the immune system and inflammation.

The third mechanism is trypsin autolysis. The trypsin molecule has two globular domains connected by a peptide chain containing an arginine at codon 122. This arginine serves as target for a second trypsin and therefore serves as a self-destruct site when R122 is exposed. Cleavage at this site permanently inactivates trypsin. Accessibility of R122 to cleavage appears to be regulated by calcium. Low calcium levels (e.g., inside the acinar cell) favor autolysis whereas high levels (e.g., inside the pancreatic duct and duodenum) prevent autolysis. Thus, trypsin activity regulates zymogen activation and calcium levels regulate trypsin survival.

Phase 2: Local Inflammation in the Pancreatic Tissue:

This phase occurs subsequent to acinar cell injury and is characterized by attraction and activation of neutrophils and macrophages in the pancreas as a result of its initial injury as well as generation and release of cytokines and other chemical mediators of inflammation. This inflammatory process is initiated by the nuclear factor-κ B (NF-κ B) pathwaywhich initially leads to the local production of Interleukin(IL)-1

and TNF-αboth in the acinar cells and local macrophages , activator protein-1(AP-1) and phosphatidylinositol-3 kinase (PI-3 kinase). (Singh et al 2001). Inhibition of these signals has been demonstrated to decrease inflammation and improve the severity of pancreatitis in most cases.. Studies of NF-κ B have demonstrated that it is activated in the pancreatic acinar cell early before the influx of inflammatory cells into the tissue(Hegyi et al.,2004).

Most studies suggest a harmful role of NF-κ B activation with one exception proposing a protective role,Blocking PI3-Kinase activation resulted in amelioration of pancreatitis in 2 rodent models without affecting NF-κ B activation. In addition to up-regulating proinflammatory cytokines, NF-κ B can increase adhesion molecule ICAM-1 effect in the pancreas(Zaninovic et al.,2000). NF-κ B is also involved in the systemic inflammatory response of acute pancreatitis.

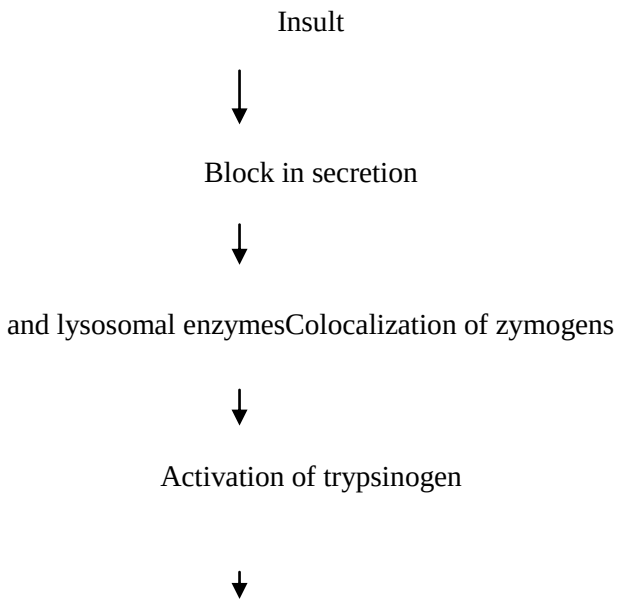
The involvement of the pancreatic enzymes themselves in the systemic inflammatory response of acute pancreatitis has been proposed. For example, pancreatic elastase but not amylase and lipase causes the pulmonary injury of the adult respiratory distress syndrome by activating NF-κ B in pulmonary tissue (Jaffray et al.,2000). Pancreatic elastase causes liver injury through increasing cytokine production in Kupffer cells utilizing NF-κ B signaling(Murr et al.,2002). During pancreatitis, there is up-regulation of an anti-inflammatory system that is probably protective. This system includes a transcriptional regulator called p8, and it is one of its regulated genes, pancreatitis-associated protein I (PAPI)(Vasseur et al.,2004,Hoffmeister et al.,2002).

Both p8 and PAPI are rapidly induced during pancreatitis. Animals with genetic deletions of p8 have a markedly augmented pancreatic inflammatory response during pancreatitis(Vasseur et al.,2004) and antibodies to PAPI injected into animals with experimental pancreatitis also markedly augmented pancreatic inflammation. These findingssuggest that PAPI is an anti-inflammatory cytokine similar to IL-10.

Pancreatic acinar cells also synthesize and release cytokines and chemokines resulting in recruitment of inflammatory cells involving different cell types (such as neutrophils, lymphocytes, macrophages, and endothelial cells) and a multitude of proinflammatory – such as IL-6, IL-8, intercellular adhesion molecule (ICAM)-1, complement components C5a , platelet activating factor (PAF), reactive oxygen species, kallikrein, nitric oxide, prostaglandins, substance P, hydrogen sulfide , neutral endopeptidase (NEP) – and anti-inflammatory mediators (e.g., IL-2, IL10).

Anatomically, these processes are characterized by inflammation and edema of the pancreatic tissue (Granger and Remick, 2005). This may be associated with vasospasm in both intra- and extra-pancreatic vessels Takeda et al.,(2005). In some cases, the microcirculation is compromised, and ischemia, hemorrhage, and necrosis may develop many of the previously

mentioned pro-inflammatory mediators have been associated with changes in the microvasculature, and platelets seem to play a crucial role.



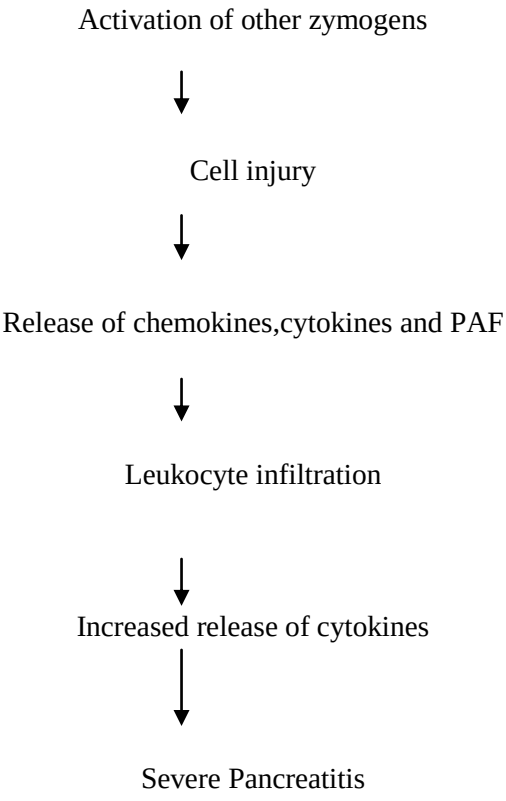


Fig.(2.2): Schematic representation of the mechanism of pathogenesis of acute Pancreatitis.Taken from (Digestion; Feb 1999; 60, ProQuest Medical Librarypg. 27).

Discussion

L-asparaginase has been an element in the treatment for acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma since the late 1960 and remains an essential component of their combination chemotherapy(Raja *et al*.,2012)..L-asparaginase primarily targets malignant lymphoblasts by depletion of the external sources of asparagines, through hydrolysis of asparagines to aspartic acid and ammonia , given the most malignant cells have limited asparagines synthetase activity, the resulting lacks of asparagines leads to apoptosis Duval et al.,(2002) and Berg , 2011) . Furthermore , L-asparaginase exhibits glutaminase activity which may contribute to the cytotoxicity of this drug (Offinan et al 2011).

One of the most serious adverse events of L-asparaginase is acute pancreatitis and it is the most common reason for stopping treatment with L-asparaginase (Kearney et al , 2009 ; Treepongkaruna et al 2009).

In our present study the apparent histopathological changes observed lightly and ultrastructurally were increase accumulation of apical zymogen granules of variable size and shape in acinar cells, edema, vacuolization and further damage in the form of focal areas of necrosis of pancreatic tissue and biochemically there is an increase of pancreatic amylase enzyme above the upper normal level also some degree of interlobular fibrosis was detected so, this pancreatitis is identified as acute pancreatitis this is in accordance with the study of (Knoderer et al 2007), two general forms of pancreatitis are recognized: acute and chronic. There is some heterogenecity within each type. The two types usually are easily distinguishable.

There are a number of experimental models of (non-alcoholic) acute pancreatitis that reproduce the responses of human disease—animal models, such as pancreatitis induced in rats or mice by administration of cerulein (CCK-8 analog), bile acid (e.g. taurocholate) or L-arginine, or by feeding mice a choline-deficient, ethionine supplemented (CDE) diet; and the *ex vivo* model of isolated pancreatic acinar cells hyperstimulated with supramaximal CCK-8 (CCK) or cerulein (CR). (Lerch and Adler, 1994 ; Pandol et al 2007).

Hashimoto et al.,(2008) in their study of cerulein- induced experimental pancreatitis in mice that acute pancreatitis increased with the number of cerulein injections. With six and nine cerulein injections, mild edema and acinar cell degenerationwere observed .With 12 ceruleininjections, the pancreas showed severe acinar cell degenerationwith significant edema and infl ammatory cell infiltration in theinterstitium . In accordance with histological changes, theyobserved a significant increase in serum amylase activity .The observed increase was proportional to the cerulein dose andthe severity of acute pancreatitis**.** The pathophysiology behind AAP is unknown (Vrooman et al., 2010) but is regarded to reflect systemic depletion of asparagines with a subsequent reduction of protein synthesis especially in organs with high protein turnover such as liver and pancreas. Information from cellular and in vivo studies as well as genetic studies in humans suggests that pathologic events that begin in the pancreatic acinar cell often initiate this disease. This cell is designed to synthesize, store and secrete the enzymes required for nutrient digestion.

Under physiological conditions, most of these enzymes particulary proteases become active only when they reach the small intestine. Drugs as L-asparaginase that cause pancreatitis result in distinct changes in acinar cell signaling. These changes initiate a sspectrum of pathologic changes within tha acinar cells that include the activation of digestive enzymes, generation and release of inflammatory and vascular mediators, changes in paracellular permeability and stimulation of cell death pathways. (Gorelick and Thrower, 2009).

Cell signaling. These changes initiate a sspectrum of pathologic changes within tha acinar cells that include the activation of digestive enzymes, generation and release of inflammatory and vascular mediators, changes in paracellular permeability and stimulation of cell death pathways.

Various in vivo and in vitro studies of experimental pancreatitis using CCK analogues such as cerulein or CCK-JMV-180 have established that the premature intraacinar activation of zymogens and increase secretion is a key event in the pathogenesis of pancreatitis followed by inhibition of secretion and retension of activated zymogens (Olegar et al. , 2001).In our study using asparaginase at a dose of 200IU, 500IU and 1000IU during the first days of experiments we observed by E/M as well as biochemically by estimation of serum amylase that the number of zymogen granules and the level of amylase increase significantly, than with further damage of pancreas with dose 1000IU and at the end of the duration of experiment (5 days), the number of zymogen granules as well as the level of serum amylase decrease significantly.

This is in agreement with the study of (Mithhofer et al., 1998) who observed activation of trypsinogen and other zymogens in the pancreatic homogenate as early as 10 minutes after supramaximal stimulation by caerulin in rats and increase over time, in addition to other markers of pancreatitis e.g. hyperamylasemia, pancreatic edema and acinar cell vacuolization can be detected at 30 minutes after supramaximal stimulation. Previous study of Grady et al 1996 strongly supports the paradigm that zymogen activation and increase exocrine pancreatic activity is the cause of pancreatitis and the autodigestion process.

Among the factors involved in inhibition of secretion and retension of activated enzymes is the loss of terminal web and its associate intermediated filaments which are believed by (Jugermam et al., 1995) to be responsible.

Another factor may also be due to disorders of exocytotic process related to SNARE proteins and small GTP binding proteins as proposed by (Gaisano et al., 2001) who explained that specific SARE proteins located on the plasma membrane and zymogen granules membranes regulate exocytosis through their interactions and a high dose CCK-8 causes displacement of one of SNARE proteins from the basal surface of acinar cell with a concomitant redirection of apical exocytosis to the basal surface. Small GTP binding proteins of Rab family have roles in exocytosis process these proteins are involved in vesicular traffic and membrane fusion in eukaryotic cells and are present in zymogen granule membranes of exocrine pancreas (Chen et al .,2004). Accumulation of large vacuoles with variable content in acinar cells is also noticed in our study and characterized by having double membrane and partially digested material, so these vacuoles have the characteristic of autophagic vacuoles which is a long noted feature of both experimental and human pancreatitis as mentioned by (Niederau and Grendell 1988 and Hirota et al .,2006).

There are 2 major hypotheses : the colocalization hypothesis (Van Acker et al, 2006) and the autoactivation hypothesis (Leach et al, 1991). According to the former hypothesis, digestive enzymes become localized with lysosomal hydrolases, such as cathepsin B and the early response of pancreatitis is the pathological, intra-acinar cell activation of digestive enzymes especially trypsinogen. Trypsinogen activation (i.e its conversion from inactive zymogen to trypsin) has been found clinically and in experimental models of acute pancreatitis and is considered a critical disease–initiating event as mentioned in the study of (Steer,1999). The latter hypothesis suggests that trypsinogen is autoactivated under low pH conditions in the

presence of serine proteases. The mode of trypsinogen delivery to the lysosomes or cellular compartments has been the subject of investigation.

There are 3 possible mechanisms for delivery of trypsinogen to the cellular compartment where activation occurs. One is fusion of zymogen granules with lysosomes crinophagy(Koike et al., 1982). The second is perturbation of normal intracellular trafficking of zymogen granules and lysosomal hydrolases. The third is endocytic vacuole formation through uptake of secreted digestive enzymes by acinar cells via endocytosis, transportation to endosomes, and fusion of endosomes with lysosomes. (Sherwood *et al*., 2007.

One important clue to distinguish between these possibilities is the appearance of cytoplasmic vacuoles within pancreatic acinar cells (Watanabe *et al*., 1984).

This is an early feature of acute pancreatitis. The nature of these vacuoles and their mechanism of formation and their relation to other pathological responses of pancreatitis have been a matter of debate. The mechanism was explained by Saluja et al 2007 due to missorting of CatB which catalyze this conversion and become colocalized with trypsinogen in unindentified compartment .

In a recent study of Hashimoto et al 2008 They found that cytoplasmic vacuoles induced in pancreatic acinar cells by experimental pancreatitis were autophagic in origin, as demonstrated by immunohistochemical studies (microtubule-associated protein 1 light chain3 expression) and electron microscopy experiments. In their experiments they used mice deficient in Arg 5, a key autophagic protein in acinar cells. Acute pancreatitis was not observed except for very mild edema in a restricted area, in conditional knockout mice, unexpectedly, trypsinogen activation was greatly reduced in the absence of autophagy.

They suggested that autophagy exerts devasting effects in pancreatic acinar cells by activation of trypsinogen to trpsin in the early stage of acute pancreatitis through delivering trypsinogen to the lysosome. The previous authors proposed that excessive autophagy is the cause of intra-acinar trypsinogen activation. In the study of (Mareninova et al .,2009) they provide evidence by using

in vivo and in vitro experimental models and both electron microscopic and immunofluorescent techniques that autophagy is activated by both pancreatitis and fasting, but, unlike fasting acute pancreatitis cause inhibition of lysosomal degradation and retardation of autophagic flux and they further found that pancreatitis impairs processing/maturation and activities of CatL and CatB, which may underlie the inefficient lysosomal degradation and their results indicate that this dysfunction, rather than missorting of CatB or excessive autophagy (Hashimoto et al .,2008), mediates the intra-acinar accumulation of active trypsin in autophagic vacuoles in acute pancreatitis. In agreement with and documented it, is the study of Gukovsky and Gukovskaya, 2010 ;Gukovsky et al 2012 who proved by several approaches using rodents as well as cell (in vitro) models of pancreatitis, in particular those induced by supraphysiological doses of cholecystokinin,the main secretagogue for acinar cells, or its analog, cerulein. They found that autophagy , the main cellular degradative, lysosome-driven process is activated but also impaired in acute pancreatitis which mediates both acinar cell vacuolation and trypsinogen activation.

The mechanisms underlying the lysosomal dysfunction in pancreatitis is abnormal processing (maturation) and activation of cathepsins, major lysosomal hydrolases; another is a decrease in pancreatic levels of key lysosomal membrane associated proteins LAMP-1 and LAMP-2 rather than blockage of autophagosome fusion with lysosomes . Their data indicate that lysosomal dysfunction plays an important initiatingrole in pancreatitis pathobiology. The impaired autophagy mediates vacuole accumulation in acinar cells; furthermore, the abnormal maturation and activation of cathepsins leads to increase in intra-acinar trypsin, the hallmark of pancreatitis; and LAMP-2 deficiency causes inflammation and acinar cell necrosis.

Thus, the autophagic and lysosomal dysfunctions mediate key pathologic responses of pancreatitis. The mainly acinar cell organellar damage observed in our present study of experimental asparaginase pancreatitis was the affection of mitochondria and rER. The mitochondria show signs of degeneration in the form of swelling, loss of mitochondrial cristae, some of them contained amorphous and electron- dense material and myelin figures.

The rER showed dilatation of their channels , these changes were similar to those observed ultrastructurally in the study of (Andrzejweska et al .,1996) on acute pancreatitis of taurocholate treated rats group. These changes may reflect sublethal changes due to ATP deficiency during

acute pancreatitis. The lack of sufficient energy supply may induce the morphological alterations such as dilatation of rER cisternae an Golgi apparatus In addition to mitochondrial morphological alterations which underlie cell organellar damage, mitochondria play a central role in regulating cell death since mitochondrial membrane permeabilization (MMP) is a universal trigger of both apoptosis and necrosis and is often considered as the point of no return in the chain of events leading to cell death (Duchen, 2004).

The molecular mediating mitochondrial permeabilization are not fully understood. Kroemer *et al* .,(2007) and (Richelli *et al*., 2011), explained that key manifestations of mitochondrial permeabilization triggering apoptotic and necrotic pathways are, respectively, the release of the mitochondria resident protein cytochrome c (as well as other apoptosis-inducing factors) into the cytosol and mitochondrial depolarization. Once in the cytosol, cytochrome c stimulates activation of specific cysteine proteases, the caspases, which mediate the downstream apoptotic events. On the other hand, loss of the mitochondrial membrane potential, ultimately leads to ATP depletion and necrosis. Thus, mitochondrial permeabilization is a central event in both apoptotic and necrotic cell death. The authors concluded that pancreatitis causes acinar cell mitochondria depolarization, mediated by the permeability transition pore (PTP) and that Genetic ablation of cyclophilin D) provide a specific tool to inactivate PTP prevents mitochondrial depolarization and greatly ameliorates the pathologic responses of pancreatitis. Further, their data suggest that mitochondrial damage, increases the demand for efficient lysosomal degradation and therefore aggravates the pathologic consequences of lysosomal dysfunction. An interesting result morphologically of our study was the presence of many acinar cells showed ultrastructural changes of apoptosis (pyknotic nuclei, cell shrinkage, prominent clumping and margination of nuclear chromatin and formation of apoptotic bodies in the group of rats receiving 500IU of L-asparaginase as well as 1000IU.

Apoptosis is the prominent feature of cell death, which is required for normal development and tissue homeostasis; it also occurs in various diseases including AP. One of the early events is shrinkage of the cell and condensation of its nuclear chromatin followed by cleavage of its DNA.

In human experimental pancreatitis, acinar cells die through both, necrosis and apoptosis Gukovskaya and Pandol, 2004).

Biochemical and morphological examination of experimental models of AP has shown that severe AP is associated primarily with necrosis and little apoptosis, (e.g. that induced by pancreatic duct ligation in the opossum, by choline deficient and ethionine supplemented diet in the mouse and by caerulin- hyperstimulation in the mouse) is associated primarily with necrosis but little apoptosis, (Gukovsyaya *et al*., 1996) whereas mild AP (e.g. that induced by caerulein in the rat) is associated primarily with apoptotic cell death and little necrosis (Bhatia, 2003).

Apoptotic cell may play a significant role in affecting mortality and morbidity in severe AP. Control of apoptosis could be a potent strategy for improvement of the clinical outcome in severe AP (Takeyama, 2005). The extent of pancreatic acinar cell apoptosis has been shown to be inversely related to the severity of the disease, so severe pancreatitis was noted to involve extensive acinar cell necrosis, but very little acinar cell apoptosis suggesting that apoptosis is a beneficial form of cell death in AP. (Kaiser et al., 1995 ; Bhatia, 2003 ; Bhatia 2004) . Recent studies of Kaiser et al. ,(1996)have found that induction of apoptosis in pancreatic acinar cells attenuates the severity of experimental acute pancreatitis. Furthermore, Kaiser et al have demonstrated that the inhibition of apoptosis by the administration of cyclohexamide was noted to worsen the severity of acute pancreatitis. Apoptosis in pancreas unlike other organs such as the involuting breast or prostate can be associated with mild evidence of inflammation such as edema and or infiltration of inflammatory cells.

This mild inflammation may indicate that apoptotic acinar cells, in contrast to other types of cells, release digestive enzymes and/or chemotactic factors, which can induce mild inflammation. Clearly, however, the degree of inflammation and, therefore, the severity of pancreatitis are significantly less in situations associated with acinar cell apoptosis than under conditions associated with acinar cell necrosis.

Conceivably, medications or other interventions that favor the development of apoptosis may minimize the severity of pancreatitis, and they could , therefore be of substantial clinical value.

Pancreatic weight was increased in our study in rats given L-asparaginase in a dose of 500IU , but no change was observed in the group of rats receiving 200 IU , followed by a decrease weight in the group receiving 1000IU. The most probably explanation was due to increase interstitial pancreatic edema observed both light and ultrastructural followed by fibroblast activation and collagen deposition. Lynne et al 1999 in their experimental studies of rats given subcutaneously injections of caerulein 24ug /kg every 8h for 2 days and the mean pancreatic weight and the pancreatic weight index were assessed by days 2,4,7 after the first injection found at day 2 no change compared to the saline controls group, by day 4 it had decreased by 42% and by 7days by 36% relative to saline controls. They interpretated their results by explaining that the reduction in pancreaticweight index was due to acinar cell loss at 2 days and was negated by stromal edema and increased connectivetissue deposition; reduction at 4 days mainly was due toreduced edema and stromal condensationseen morphologically as closer approximation of the epithelial tissue elements.

Even though the pancreas had a relatively normal histological appearance at 7 days, its weight remained markedly reduced compared with saline controls, indicating regeneration was incomplete,10 days or so usually being required for full recovery. Destruction of the cell membrane, with release of pancreatic enzymes into the interstitial space, is a typical finding in human acute pancreatitis.(Kloppel *et al* ,1986).

In our study we observed hyperamylasemia (increase in serum amylase) with L-asparaginase 500 and 1000IU. Muller et al ., 2007 using immunohitochemical tracers methods hypothesize that very early in the onset of the disease, amylase and other pancreatic enzymes leak out of the acinar cells through basolateral and basal cell membrane disruptions that allow albumin and IgG to penetrate into acinar cells as well as Ca^{2+} ions and exit of molecules such as enzymes. Amylase is normally confined within membranes, rather than being free in the plasma of the pancreatic cell.

To be detectable in the blood, amylase must also enter the vascular compartment. During caerulin induced pancreatitis, changes in the pancreatic vessels aresimilar to the changes in acinar cells, with cytoplasmic vacuoles in the endothelial cells and perivascular edema (Gress *et al*., 1990). Previous reports have shown that hyperstimulation by infusion of cerulein leads to hyperamylasemia within 30 minutes. Adler et al , 1978 ; Watanabe et al 1984 ; McEntee et al

1989 In addition to acinar cell damage and elevated pancreatic enzymes minor intercellular edema was observed in all groups given L-asparaginase at different doses.

The role of the microvasculature, and especially alterations in the microvasculature that lead to the development of acute pancreatitis, has been emphasized by different authors. There is evidence from experimental models that pancreatitis is associated with vascular disorders such asreduced pancreatic flow, increased capillary permeability, and vascular leakage. (Klar *et al* .,1990) .

Ultrastructural alterations in acinar cells have been reported after 15 minutes, but preceded microvascular changes. (Kelly *et al*., 1993), Mononuclear cell infiltration was also detected in our study and the release of cytokines and chemokines by these cells trigger the inflammatory response observed in the form of focal necrotic areas.

Multiple therapeutic modalities have been suggested for AP, but none have been unambiguously proven to be effective yet, and to date the treatment is essentially supportive. Various experimental models of AP were used for the evaluations of novel drugs. However, the complex pathophysiology of the disease, which is still ill-defined, and the reality that numerous etiologic factors can initiate the disease through diverse mechanisms hinders the development of efficacious specific treatment. (Greenberg *et al*., 2000). The development of the long acting somatostatin analogue, octreotide, introduced in the early 1980s has led to its use in experimental pancreatitis as well as in clinical patients , its longer half life allows subcutaneous administration avoiding the need for continuous intravenous infusion (Pless *et al*., 1986) . It is conventional wisdom that 'to rest the gland' is beneficial in the management of acute pancreatitis. Although the validity of this assumption has led to clinical trials with nasogastric suction, (Naeije *et al* , 1978) cimetidine, (Broe *et al* , 1982) and glucagon (Durr *et al*, 1978) in the treatment of acute pancreatitis So, in clinical in clinical trials would be expected to cause a 70% reduction in exocrine pancreatic secretion. (Gullo et al 1987) This may explain the successful use of somatostatin analogues in the treatment of pancreatic fistulas and pseudocysts. (Gullo and Barbara, 1991).

In our after using octreotide with different doses of L-asparaginase we observe improvement in pancreatic histology with dose 500IU as well as decrease zymogen granules observed ultrastructural in pancreatic acinar cells mitochondria restore its normal shape. This in agreement

with the study of (Adler *et al*., 1980; De Rai *et a*l .,1988) who foun histological damage with somatostatin given i.v. started immediately after induction of pancreatitis.

Previously Baxter etal 1985 in addition to show a histological benefit with somatostatin treatment found a dramatic improvement in survival of rats after duct ligation.even if treatment was started immediately or delayed for 24 hours after duct ligation. These results suggest that differencesin duration of treatment, but not in timing of treatment or size of dose, may explain some of the differences in results obtained. Augelli et al 1989 were able to show a reduction in histological severity inanimals given octreotide, 5, ug/h, before the induction of pancreatitis although no effect was seen if treatment was delayed until pancreatitis was established despite continuing treatment for 24h. In the study of Diavliakos *et al* 1990 an ex vivo model was used and histological benefit was seen after only 1 injection of octreotide even if treatment was delayed until one after the induction of pancreatitis.

Zhu *et al*., (1991) giving octreotide 2 ,ug/kg/h showed a reduction in histological severity in a rat bile injection model. Once again, this effect was only seen if treatment was commenced before the induction of pancreatitis. Kaplan et al 1996 reported in their study of acute experimental pancreatitisThey report that octreotide ameliorated pancreatic edema and histopathological injury score but have not observed any effect of octreotide treatment was begun at different times no deadline to begin octreotide treatment. Although (Chen *et al*,. 1998) stated that deadline to begin octreotide is 24 hours.

Murayama et al,. 1990 giving octreotide immediately showed beneficial effects on both hitopathological and biochemical parameters in experimental pancreatitis . Less pancreatic edema, necrosis and inflammatory cell infiltration, reduction in serum amylase were also observed. Results from clinical studies show evidence of a trend towards a reduction in death and complications rate with octreotide although no sufficiently large trial has yet been performed for firm conclusions to be drawn on the efficacy of octreotide in acute pancreatitis and in clinical setting there is almost always a delay between the beginning of the pathologic process and initiation of treatment.

Paran *et al*., (1995) repoted positive results for octreotide treatment in a small patient population with severe acute pancreatitis. The authors found significantly decreases in septic complications (74vs 26%, p=0.004) and in the development of adult respiratory distress syndrome (ARDS) (63 vs 18%) resulting in a significantly shorter hospital stay and the mortality rate was lower in the group treated with octreotide. Similar findings were reported by Fiedler et al , (1996)involving 39 patients with severe acute pancreatitis. At a dose of 100 ug t.i.d.octreotide significantly reduced the frequency of ARDS (40vs18%) , circulatory shock and mortality. As acute pancreatitis is the most common complication after endoscopic retrograde cholangiopancreatography (ERCP) occurring in up to 11% of cases although asymptomatic hyperamylasaemia occurs in up to 70%. Octreotide have been evaluated in the prophylaxis against acute pancreatitis after ERCP. The proposed mechanisms of action of octreotide in acute pancreatitis in addition to inhibition of pancreatic secretion is the stimulation of the phagocytic cells of the reticuloendothelial system. In experimental acute pancreatitis, survival can be improved by stimulation of the reticuloendothelial system with either zymosan or glucan. (Browder *et al*., 1987). Conversely, depression of the reticuloendothelial system results in worsened survival. There is also some evidence of reticuloendothelial system depression in patients with acute pancreatitis. (Banks *et al*., 1991). In our study we found decrease in inflammatory cells and mononuclear cells infiltration, since octreotide has been known to have an anti-inflammatory activity by reducing the inflammatory reactions and the release of inflammatory mediators such as TNF-α , IL-1β and IL-6, oxygen free radicals, thus prevent the progression of acute pancreatitis from mild to severe form and block the progression of systemic inflammatory response syndrome and avoid multiple organ dysfunction .

The pathogenesis of SAP has also been further recognized in recent years The intestinal mucosal barrier is injured during SAP mainly by bacteria,

and endotoxin translocation through mesenteric lymph node, thoracic duct and systemic circulation, leading to secondary infection or even systemic inflammatory response syndrome (SIRS), inducing multiple organ dysfunction syndrome (MODS) and resulting in death. Octreotide is one of the common SAP medications at present.(Czako *et al*., 2004 ; Suzuki *et al*., 2008).

As a common used drug to treat SAP, octreotide provide good protection of multiple organs in SAP rats. The underlying mechanisms include decreasing the release of inflammatory mediators,

inhibiting inflammatory reaction, stabilizing cellular structure,reducing cell necrosis, improving microcirculation, and intensifying immunity . It also alleviated injury to the intestinal mucosa and improved the survival of SAP rats. Concerning the role of octreotide as a therapeutic agent in SAP by promoting pancreatic acinar cell apoptosis , proved in their study on mice that after administration of SSa (Somatostatin secretagogue analogue) the apoptotic index is markedly increased as well the expression of apoptosis regulated gene bax which is the promoter to aacelerate the pancreatic acinar cell apoptosis , these findings support the concept that apoptosis might be a beneficial response to acinar cell injury in pancreatitis .

CONCLUSION

Although ASNase by itself did not cause pancreatitis, it did cause increased levels of pancreatic enzymes and histological damage to the pancreas associated with pancreatic injury or prepancreatitis. Prior treatment with octreotide prevented the development of ASNase-induced pancreatic injury.

RECOMMENDATIONS

-Pay attention of clinicians and practitioners to use octreotide as prophylactic before onset of pancreatitis and subsequent permanent irreversible damage of the gland in high risk individuals.

-Even in severe acute pancreatitis which is one of the huge threat to human health with many complications and with mortality rate ranges from 11.8% to 25% inducing pathological changes not limited to the pancreas but extend to extrapancreatic organs causing multiple organ dysfunction syndromes, octreotide was the drug of choice for the protection of these organs due to its extensive pharmacological effects and actions

-Further studies to clarify the mechanisms underlying its actions are needed in large controlled clinical trial to confirm its use as prophylactic against acute pancreatitis in routine clinical practice.

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