INTRODUCTION

The word leukemia comes from the Greek words 'leukos' which mean white blood and refers to excess white blood cells in the blood.[1] The credit for the discovery of leukemia goes to the ancient Greeks, who recognized this blood disease way back in the 4th century BC.[2]

Acute lymphoblastic leukemia (ALL) is a malignant disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow, resulting in a marked decrease in the production of normal blood cells. The lymphoblasts proliferate in organs other than the marrow, particularly the liver, spleen and lymph nodes.[3]

Leukemia is the commonest pediatric cancer accounting for about 30% of all childhood cancers. With an incidence expected to reach up to 4.75 cases per 100.000 people worldwide, ALL represents 80% of all leukemia diagnoses.[1,4] ALL incidence is higher in males than females with M: F incidence ratio of 1.2:1. There is a striking incidence peak at age 2-7 years.[5]

According to the national cancer registry (NCR) Egypt, leukemia is the leading cause of malignancy in children, constituting 36.7% of all cases of childhood cancer diagnosed annually.[6]

Survival rates from leukemia in the United States have been improved significantly over the period from 1977-2001. ALL five years overall survival increased from 68% during the year 1977-1981 to 86% during 1997-2001.[7] The pre-B cell ALL accounts for the majority of ALL cases (80%) while about 15% of cases consist of pre-T cell type.[8]

Although a significant improvement in both ALL diagnosis and treatment has been made over the past decades; the etiology of most cases of ALL remains unknown. Confirmed clinical and epidemiologic associations explain less than 10% of childhood leukemia incidence, leaving at least 90% of cases with an unresolved etiologic mechanism.[9-10]

The difficulty arises from the fact that pediatric leukemia, like most cancers, has multifactorial etiologies involving the interaction between various aspects originating from the environment as well as human genetics.

Environmental factors: established evidence for increased risk of ALL includes sex, age, race, prenatal exposure to x-rays, therapeutic radiation and specific genetic syndromes.[10,11] Ionizing radiation exposure through in utero diagnostic x-ray is an established risk factor for childhood ALL but it likely contributes for very few if any cases presently due to decline of dosage and frequency of use during pregnancy.[12]

Children who are treated for a prior malignancy especially with treatments including alkylating agents are at increased risk of developing leukemia.[13] Maternal age greater than 35 yeas at the time of child birth appears to be associated with increased risk of childhood leukemia.[14,15] There is also suggestion that history of maternal fetal loss (stillbirth or miscarriage) is associated with a slightly increase risk of childhood leukemia.[16]

Toxic exposure in utero to maternal Epstein-Barr virus (EBV), reactivation of other viral entities such as Cytomegalovirus and Herpes simplex virus has been proposed as a causative factor in some infants and children with ALL.[17]

An extensive review of ALL in children by Buffler et al detailed variable potential hazards: occupational exposures in parents to aromatic hydrocarbons, household chemicals, pesticides, diet, and infections.[10] Numerous potential predisposing features studied or established include positive associations with the specific genetic syndromes, high birth weight,[18] ethnicity,[19] and maternal smoking.[20]

Interestingly, significant negative association was observed between breastfeeding for more than 6 months and incidence of leukemia. This may be explained by the fact that breastfed infants are exposed to infectious agents transferred from the mother's milk, thereby leading to early exposure to infectious organisms and development of natural antibodies.[21-23]

Genetic factors are presumed to play a significant role in the cause of ALL. Evidence for this is based on several observations including the association between various constitutional chromosomal abnormalities and childhood ALL.[24]

Current evidence suggests that leukemia results from chromosomal alterations and mutations that disrupt the normal process by which lymphoid or myeloid progenitor cells differentiate.[25-27] The underlying triggers for molecular damage may be inherited during pregnancy and may develop during infancy and childhood.

Chromosomal translocations are a "hallmark" genetic event in leukemia. Many leukemia patients have a chromosomal translocation that is often the only observable cytogenetic aberration.

An increased risk of ALL has been also associated with certain congenital syndromes and chromosomal abnormalities (Down syndrome), genetic instability (ataxia telangectasia, Fanconi anemia), Specific genotypes of certain polymorphic enzymes that may play a role in detoxification or DNA repair process have been linked to the risk of ALL.[28]

The general mechanisms underlying the induction of ALL include the aberrant expression of proto-oncogenes, chromosomal translocations that create fusion genes encoding active kinases and altered transcription factors, and hyperdiploidy involving more than 50 chromsomes. These genetic alterations contribute to the leukemic transformation of hematopoietic stem cells or their committed progenitors by changing cellular functions they alter key regulatory processes by maintaining or enhancing an unlimited capacity for self-renewal, subverting the controls of normal proliferation, blocking differentiation, and promoting resistance to death signals (apoptosis).[29]

The most common structural genetic abnormality in childhood leukemia is a fusion of two genes, TEL and AML1. It is generated by simultaneous breaks in chromosomes 12 and 21 followed by chromosome translocation forming a chimeric or fusion gene as shown in Fig (1). Each patient's leukemic cells have a unique (or clone specific) breakpoint in the DNA sequence that can be identified by PCR technique.[30]

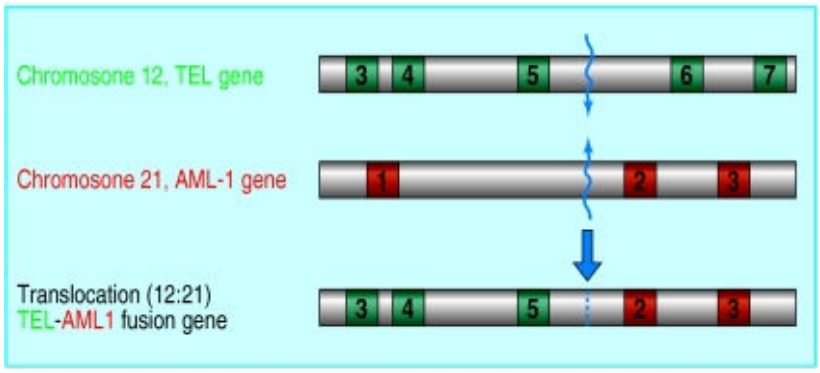

Figure1: The TEL-AML1 translocation.

The figure shows the genes break in non-coding (grey) regions between the coding regions (numbered, green or red), and re-joining of the two broken genes forms a novel fusion gene.

A common chromosomal translocation in infant leukemias is MLL-AF4 fusion gene. Such Chromosomal translocations are believed to be often the initiating event of leukemia, occurring during fetal development. This evidence is supported by the fact that concordance rates of leukemia in infant twins are exceptionally high, approaching 100%.

However, for twins aged 2-6 years with acute lymphoblastic leukemia the concordance rate is considerably lower, around 5%. It still represents a 100-fold extra risk of leukemia, but also indicates the need for some additional postnatal event(s). Greaves[30] proposed a "two hit" model for the natural course of childhood leukemia. Thus, natural history of pediatric leukemia, involves pre-natal initiation of preleukemic clones (frequently by chromosomal translocation) followed by secondary postnatal mutation and overt disease (with a latency period ranging from few months to up to 15 years). He also proposed a 'delayed-infection' hypothesis where infection was suspected to have a crucial role in promoting, through the immune response, the second or postnatal genetic error, or 'hit'.[30,31]

Diagnosis of Acute Lymphoblastic Leukemia

Clinical features of ALL could be categorized into general nonspecific manifestations, hematologic manifestations and manifestations due to invasion of different body organs as shown in table (1).[32,33]

When clinically suspected the diagnosis of leukemia requires several diagnostic tests to ascertain the disease, determine its extent and classify into its different subtypes. Initial **complete blood count** with differential WBCs count and microscopic examination of the blood film is mandatory. It commonly shows moderate to marked hemoglobin reduction and thrombocytopenia. White blood cell count may be low, normal, or increased. Blood smear usually shows blasts cells (may be absent with very few to absent in cases with leucopenia).[32]

Routine **laboratory and imaging studies** are undergone for full assessment of the patient prior to initiation of treatment, to detect any organ dysfunctions, complications, and as a baseline for further follow up. They include blood chemistry (for electrolytes, blood urea, uric acid, liver function tests, LDH), chest radiograph, abdominal ultrasonography, coagulation profile, cardiac function monitoring (electrocardiogram and echocardiogram) and infectious disease profile (Varicella, cytomegalovirus, herpes simplex, and hepatitis antibodies screening).

Gold standard of leukemia diagnosis is "**Bone marrow examination**". Normal bone marrow shows less than 5% blast cells (M1 marrow). Blast cell is a relatively undifferentiated cell with diffusely distributed nuclear chromatin, one or more nucleoli, and basophilic cytoplasm. In leukemic patients, normal bone marrow structures are replaced by blasts; usually up to 80–100%. By arbitrary convention, more than 25% blasts (M3 marrow) is required to confirm the diagnosis of acute leukemia.[33]

Special bone marrow studies that help in classification of the subtype of leukemia include the following:

- **Histochemical studies** distinguish cytochemical characteristics of blast cells in various types of leukemia.
- **Immunophenotyping** using flow cytometry, which is a technique that uses a panel of monoclonal antibodies to specific surface markers expressed on blast cells. It permits the distinction of the different immunologic subclones of leukemia. The panel of used antibodies should include at least one marker that is highly lineage specific, for example, CD19 for B lineage, CD7 for T lineage, and CD13 or CD33 for myeloid cells.[32]

Table 1: Clinical features of childhood acute lymphoblastic leukemia:

General systemic manifestations	Fever (most common symptom in about 60 % of patients), lassitude, pallor, and bone pain.
Hematologic effects	• Anemia (in about 90% of patients at diagnosis) manifested by pallor, tachycardia, easy fatigability, up to heart failure. • Neutropenia manifested by fever and recurrent infections. • Thrombocytopenia leading to bleeding manifestations. • Pancytopenia (2% of patients at diagnosis)
Invasion of body organs and systems	**Signs and symptoms**
Lymphoid system	In about 50 to 60 % of cases Lymphadenopathy, splenomegaly and hepatomegaly
Central nervous system (CNS)	occurs in less than 5% of children • Raised intracranial pressure (e.g., morning headache, vomiting, papilledema, bilateral sixth-nerve palsy) • Parenchymal involvement (e.g., focal neurologic signs, cranial nerve palsies, convulsions, cerebellar manifestations) • Hypothalamic syndrome (polyphagia, hirsutism and behavioral disturbances) • Diabetes insipidus. • CNS hemorrhage
Genitourinary tract	• Renal involvement (hematuria, hypertension, and renal failure). • Testicular Involvement, either overt or occult, and priapism. • Ovarian involvement.
Gastrointestinal tract	Bleeding is the most common manifestation of GIT involvement especially if concomitant severe thrombocytopenia.
Bone and joint	Bone pain is one of the initial symptoms in 25% of patients (due to direct leukemic infiltration of the periosteum, bone infarction, or expansion of marrow cavity by leukemic cells)
Heart and lungs	• Symptomatic heart disease occurs in less than 5% of cases. • Lung involvement may be due to leukemic infiltrates (especially in cases with hyperleukocytosis) or hemorrhage.
Eyes	Occult ocular involvement seen on careful ophthalmologic investigation in up to one-third of newly diagnosed patients. Retinal hemorrhage, ocular nerve palsies, and papilledema.

- **Cytogenetic analysis** of leukemic blasts provides clinically relevant information for diagnosis and prognosis of childhood leukemia. There are two major classes of cytogenetic aberrations; those that result in the visible loss or gain of chromosomal material and those that result in a balanced exchange without apparent loss or gain of DNA. Fluorescent *in situ* hybridization (FISH) and polymerase chain reaction (PCR) are employed for detection of these genetic defects with high sensitivity level. Table (2) shows common cytogenetic abnormalities found in leukemic clones.[33]

Table 2: Cytogenetic abnormalities in acute lymphoblastic leukemia:[33]

Structural chromosome changes in B-ALL	t(9;22)(q34;q11)BCR-ABL fusion; Philadelphia chromosome* Cryptic t(12;21)(p13;q22)*TEL/AML1*# t(1;19)(q23;p13)* t(8;14)(q24;q32) and variant MLL rearrangements (11q23) t(4;11)(q21;q23)* t(6;11)(q27;q23) t(9;11)(p12;q23) t(10;11)(p12;q23) t(11;19)(q23;p13.3)
Structural chromosome changes in T-ALL	t(10;14)(q24;q11) t(7;10)(q35;q24) t(1;14)(p15;q11) t(7;9)(q34;q32) t(11;14)(p15;q11)20p t(11;14)(p13;q1) t(7;11)(q35;p13) t(8;14)(q24;q11)
Numerical chromosomal abnormalities	Hypodiploid Hyperdiploid (47-49 chromosomes)** Hyperdiploid (>50 chromosomes)# Near triploidy Near tetraploidy*
* Known to carry poorer prognosis. #confers favourable prognosis. **most frequent numerical chromosomal abnormality in ALL identified in over 40% of cases of precursor B-cell disease.	

Cerebrospinal fluid examination is mandatory to assess infiltration of CNS. Cerebrospinal fluid findings for the diagnosis of CNS leukemia require presence of more than five WBC/mm3 and identification of blast cells on cytocentrifuge examination using cytopsin stain. CNS involvement is classified according to the number of WBCs and blasts on cytocentrifuge slide as CNS 1 <5 WBCs/mm3 and no blasts on cytocentrifuge slide, if blasts are present on cytocentrifuge slide it is classified as CNS 2 <5 WBCs/mm3 or CNS 3 ≥5 WBCs/mm3.[32]

Classification of Acute lymphoblastic leukemia

Morphologic classification was issued by French-American-British (FAB) cooperative group in 1976. It classifies leukemia subtypes as, **L1 subtype** which accounts for 85% of children with ALL and is characterized by small cell predominance; **L2 subtype** which consists mainly of large varied cells, accounting for 14% of children with ALL; and lastly **L3 subtype** which shows large varied cells with vacuoles, it accounts for 1% of children with ALL and is treated as Burkitt's lymphoma.[34]

Immunophenotypic classification is based on markers expressed by leukemic clones. ALL is differentiated into: **Pre-B-cell ALL**, accounting for 80% of pediatric ALL cases, and is further subdivided based on cytoplasm Ig into transitional pre-B or common ALL antigen (CALLA positive). **Mature B-cell ALL** is much less common and accounts for 1-2% of ALL cases. Lastly, **T-cell ALL** accounts for 15-20% of cases.[32]

Morphologic, immunologic and cytogenetic (MIC) classification of acute lymphoblastic leukemia has also been proposed, as a practical classification of the acute leukemias as it includes several characteristics of prognostic value, table (3).[33,35]

Table 3: MIC classification of acute lymphoblastic leukemia:[33]

		MIC group class					
		Early B-precursor ALL	Common ALL	Pre-B ALL	B-cell ALL	Early T-precursor ALL	T-cell ALL
FAB (Morphologic characteristics)		L1, L2	L1, L2	L1	L3	L1, L2	L1, L2
Immunologic markers	CD2					+	+
	CD7	-	-	-	-	+	+
	CD10*	+	+	+	±		
	CD19	+	+	+	+	-	-
	TdT	+	+	+	-	+	+
	*c*Ig	-	-	-	+		
Karyotype		t(4;11), t(9;22)	6q–, near haploid, del(12) or t(9;22)	t(1;19), t(9;22)	t(8;14), t(2;8), t(8;22)	t/del(9p)	6q–

* CD10 is usually negative in cases of t(4;11) and positive in cases of t(9;22)

+: positive, –: negative, no symbol: not specified by MIC workshop. FAB: French–American–British classification; TdT: terminal deoxynucleotidyl transferase.

Lately, in 2008, the World Health Organization (WHO) has developed a new classification of ALL based on cytogenetic and molecular characteristics, table (4).[36]

Table 4: WHO Classification of ALL:[36]

B lymphoblastic leukemia/lymphoma
B lymphoblastic leukemia/lymphoma, not otherwise specified (NOS)
B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); BCR-ABL 1
B lymphoblastic leukemia/lymphoma with t(v;11q23); MLL rearranged
B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) TEL-AML1 (ETV6-RUNX1) B lymphoblastic leukemia/lymphoma with hyperdiploidy
B lymphoblastic leukemia/lymphoma with hypodiploidy
B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) IL3-IGH
B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);TCF3-PBX1
T lymphoblastic leukemia/lymphoma

Risk Stratification of ALL patients

Risk stratification of leukemia patients is done at diagnosis to determine the protocol of treatment to be implemented as shown in table (5).

Table 5: Risk stratification according to Modified CCG protocols used at AUCH:

	Standard Risk	High risk
1. Age	>1 years and <10 years	≤1 year or ≥10 years
2. White cell count	< 50.000	> 50.000
3. Immunophenotype	Early pre-B-cell ALL has the best prognosis	Mature T-cell ALL has a worse survival
4. DNA index	>1.16 Hyperdiploid	≤1.16 Hypodiploid
5. Cytogenetics	Trisomies of chromosomes 4, 10, and 17	Translocations involving the MLL rearrangement on 11q23Philadelphia chromosome t(9;22)(q34;q11)
6. CNS & testicular disease	No CNS disease at diagnosis	Presence of CNS disease at diagnosis or overt testicular disease

Prognostic Factors

Only 35 years ago, less than 1% of children with ALL were expected to be long-term survivors. However, today approximately 80% of children and adolescents with ALL are cured. Furthermore, 20-30% of children with leukemic relapse have a chance of cure with second-line treatment.[33]

Several important prognostic factors have already been identified, such as age, initial WBCs count, early response to treatment, cytogenetics, etc... (Tables 2 and 5). These factors are applied to stratify children into different treatment groups according to their relative risk of treatment failure. However, techniques that are more accurate are still required for decision taking regarding intensity of treatment to be given to each patient. One of the techniques that could further add as a prognostic factor is the quantification of minimal residual disease (MRD) of leukemic patients at different stages of treatment.[32,33, 37,38]

Minimal Residual Disease and Its Implication in the Management of Leukemia[32,33,39]

The extent of clearance of leukemic cells from the blood or bone marrow during the early phase of therapy is an independent prognostic factor in ALL. Remission status in leukemia used to be determined based on morphologic findings alone. However, methods with increased sensitivities have emerged for the detection of minimal residual disease (MRD).

The most commonly used are flow cytometric detection of aberrant immunophenotypes and polymerase chain reaction analysis of clonal antigen-receptor gene rearrangements. Flow cytometry for leukemia-associated immunophenotype can detect a leukemic cell among 103-104 normal cells. PCR techniques based on chromosomal aberrations with known breakpoints have a detection limit as low as one cell among 104-106 cells. When applied together, these techniques can monitor MRD in virtually all cases of ALL.

Patients with low (less than 0.01% leukemic cells in bone marrow) or absent MRD after completion of induction therapy appears to have a good outcome. Sequential monitoring of MRD can further improve its clinical utility as a risk assessment tool as steady decrease of MRD levels during treatment is associated with a good prognosis. On the other hand, patients with positive MRD $\geq$ 0.01% or increase in MRD positivity seem to carry a poorer outcome with higher tendency to clinical relapse.

Treatment protocols may be modified, depending on the level of MRD, thus sparing children with predicted good outcome from more adverse effects of chemotherapeutic regimens and reserve the more intensified regimens for children with poor prognostic criteria, reducing rates of relapse and augmenting chances of cure from disease.

Treatment of Acute lymphoblastic leukemia

Treatment of ALL consists of **general supportive care** commonly needed at presentation, and **Specific Therapy**, aiming at cure (complete remission) from the disease.

A "**complete remission**" is defined as absence of any symptoms attributable to the disease, normal CSF examination, a normal peripheral blood picture ($\geq$500/mm3 granulocytes, 75,000/mm3 platelets, and 12 g/dl hemoglobin with no blast cells) and a moderately cellular bone marrow with less than 5% blast cells.[32]

There are four basic phases in the design of ALL treatment protocols. These phases include remission induction, treatment of clinical or occult CNS disease (consolidation), intensification, and maintenance (continuation) therapy.[33]

Most commonly used drugs in treatment of pediatric ALL are steroids (dexamethasone or prednisolone), vincristine, L-asparaginase, methotrexate, adriamycin, cyclophosphamide, cytarabine, and 6-mercaptopurine. These drugs give different degrees of immunosuppression. Cyclophosphamide is probably one of the most potent immunosuppressive among these drugs. Adriamycin is also a highly myelosuppressive drug and has a cumulative cardiotoxicity. Steroids are lympholytic through the activation of nucleases leading to DNA fragmentation (apoptosis). It is important to note that altered prognosis of ALL is mainly the result of very intensive chemotherapy associated with severe suppression of both the normal hematopoietic stem cells and the immune system.[33]

Complications of leukemia and antineoplastic treatment

Major complications **(Oncologic emergencies)** are expected mainly in children who present with high leukemic cell burden and hyperleukocytosis (WBC $\geq 100\times10^3$cell/mm^3). They include **Tumor lysis syndrome**, a life-threatening metabolic complication that results from either spontaneous or chemotherapeutically induced leukemic cell death.[40] **Hyperleukocytosis/leukostasis syndrome** is due to microcirculation impairment by intravascular clumping of leukemic blasts, resulting in local hypoxemia, endothelial damage, hemorrhage and infarction. **Superior vena cava syndrome**, may occur in children with mediastinal mass.[41]

Bleeding, usually occurs due to thrombocytopenia resulting from marrow infiltration and/or chemotherapy induced marrow aplasia, disseminated intravascular coagulation and septicaemia.[33] **Thrombosis** particularly venous thrombosis may occur especially with L-asparaginase use.[42] **Infections** represent the second most common cause of death in leukemia patients after the disease itself. They most commonly occur during periods of neutropenia. Viruses are the most common causes of infection. Bacteria are responsible for most life-threatening complications as well as invasive fungal infections, especially Candida and Aspergillus species.[43]

The improved survival of childhood ALL has focused attention on the **late complications** of antileukemic therapy, shown in table (6). Systematic ongoing follow-up of these patients is hence important for early detection and intervention for these potentially serious late-onset complications.[33,44]

ALL relapse[32]

Relapse is diagnosed by the appearance of any of the following in a leukemic patient during or after he ends his treatment:

- More than 50% blasts in a single bone marrow aspirate.
- Progressive repopulation of blasts in excess of 5%, culminating in more than 25% in two or more bone marrow samples separated by 1 week or more.
- More than 25% blasts in the bone marrow and 2% or more circulating lymphoblasts.
- Leukemic cell infiltration in extramedullary organs, for example, CNS or gonads (biopsy proven) (for the diagnosis of isolated extramedullary relapse, the bone marrow should contain less than 5% blasts).
- Blasts in the CSF with a cell count greater than five WBC/mm^3.

Table 6: late sequelae of cancer treatment:[33]

Organ	Affection	Aetiology*
Gonads	Infertility	Alkylating agents, radiotherapy
Liver	Fibrosis/cirrhosis	Methotrexate, mercaptopurine, hepatitis B, C virus
	Hepatocellular carcinoma	Hepatitis B virus
	Venoocclusive disease	Busulfan, bone marrow transplantation
Lung	Fibrosis/pneumonitis	Busulfan, radiotherapy
Kidney	Tubulopathy (Fanconi syndrome)	Ifosfamide
Thyroid	Tumors, hypothyroidism	Radiotherapy
Spleen	Overwhelming sepsis	Splenectomy/radiotherapy
Bone	Osteonecrosis	Steroids
Heart	Cardiomyopathy	Anthracyclines, radiotherapy
Central nervous system	Leukoencephalopathy, ↓IQ, ↓cognition, ↓Psychomotor skills, ↓Growth	Radiotherapy, intrathecal chemotherapy
	Second malignancies	Genetic predisposition; alkylating agents, epipodophyllotoxins

* Some of these drugs are not used in ALL patients.

Immunity and leukemia

Both short-term (<2 years) and long-term immunologic effects of chemotherapy have been documented in children treated for leukemia. All arms of the immune system appear to be affected in these children.[45,46]

Regarding innate immune system, leukemic patients may have various disruptions of the mucocutaneous integrity providing a key portal of entry for bacterial and fungal pathogens (because of invasive procedures, use of vascular catheters, irradiation, or cytotoxic chemotherapy). Several antineoplastic drugs may induce severe mucosal disruption (as high-dose methotrexate, cytosine arabinoside, and etoposide). GI barriers may also be disrupted by mucosal disruption due to Herpes simplex virus (HSV) or cytomegalovirus (CMV) and permit bacteria and/or fungi translocation from the mesenteric capillary bed and portal venous system. Moreover, underlying disease, exposure to broad-spectrum antibiotics and prolonged hospitalizations contribute to colonization by more pathogenic bacteria replacing normal bacterial flora. Nutritional deficiencies also affect B and T lymphocytes, PMNs, mononuclear phagocytes, complement system function, and cytokine immunoregulation.[45]

Phagocytic cells may be quantitatively or qualitatively impaired leading to susceptibility primarily to bacterial infections (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and wide range of resistant gram-negative organisms) and fungi (particularly Candida spp., Aspergillus spp., Fusarium spp., and Zygomycetes). Quantitative declines of PMNs and MNCs occur commonly through pharmacologic immunosuppression. Moreover, qualitative abnormalities of PMN function can occur, including suboptimal chemoattractant responsiveness, bactericidal activity, and superoxide production.[45]

During the course of chemotherapy, there may be substantial qualitative and quantitative defects in humoral immunity and antibody response. Defective immunoglobulin synthesis (more marked for IgG and IgM compared to IgA) leads to an increase in susceptibility to encapsulated bacteria, particularly Streptococcus pneumoniae, Haemophilus influenzae type B (HIB), and Neisseria meningitides.[45,47-50]

In normal children, immunization with live viral vaccines such as those against measles and rubella as well as recombinant vaccine against HBV results in production of specific antibodies (immunoglobulins). The long-term antibody production is maintained by bone marrow (BM) plasma cells. In leukemic children, the proportion of plasma cells in the BM at diagnosis is decreased due to an infiltration by leukemic cells. Moreover, during treatment, plasma cells in the BM are depleted due to direct cytopathic effects of chemotherapy that greatly disturbs the microenvironment in the BM. However once chemotherapy is ended, a new pool of plasma cells is re-established in the BM and decrease in levels of immunoglobulins is usually normalized within one year after treatment.[46]

Regarding cellular immunity in leukemic children, intensive chemotherapy is associated with a near-total loss of CD4 T-cells (helper), which do not reappear until several months after the end of treatment. Patients are therefore prone to fungal, viral, and intracellularly replicating bacterial pathogens (e.g., Mycobacterium tuberculosis, Listeria monocytogenes, and Salmonella species). However, they retain a T-cell compartment that is enriched for T-cell memory. This implies that strategies to expand antigen-specific T-cell responses to infectious pathogens or tumor antigens may be most successful if they target memory T cells in cancer patients.[45,51]

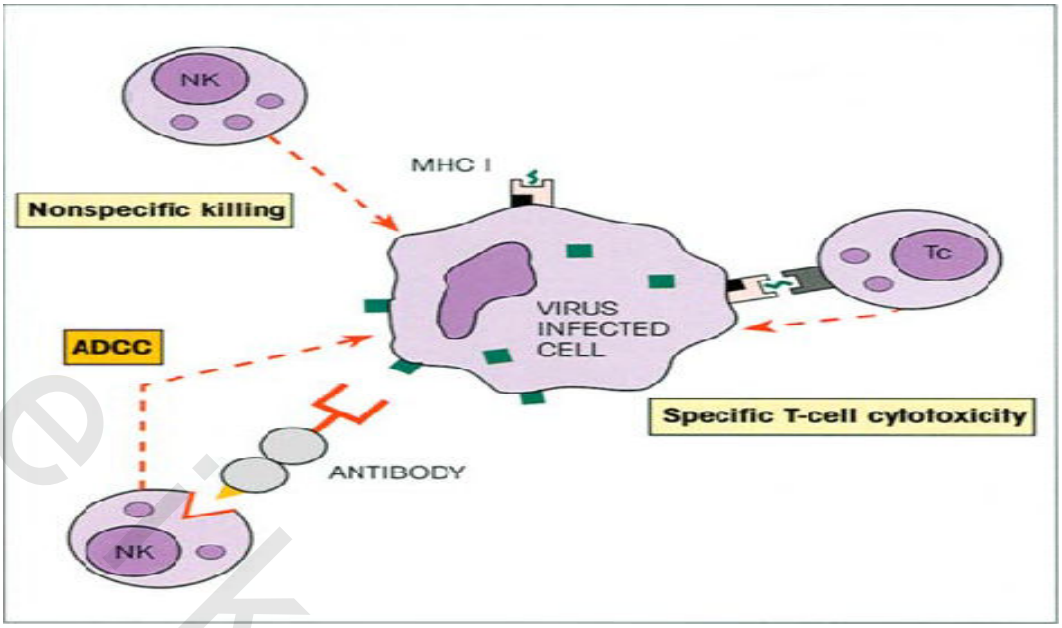

Figure 2: Killing of virally infected cells.[52]

In clinical settings, infections are an important complication of intensive polychemotherapy, especially during granulocytopenic episodes, and a common cause of death. Most are presumably bacterial, but often a significant microorganism cannot be found by standard methods. Therefore, any febrile child with leukemia and an absolute neutrophil count $<0.5\times10^9$cell/L should be considered bacteremic and treated with broad-spectrum antibiotics covering Gram-positive and Gram-negative organisms.

A variety of nonbacterial opportunistic microorganisms can invade the immunocompromised host. Fungal infections, especially *Candida* and *Aspergillus* species, are increasingly observed during prolonged periods of immunosuppression. Pneumocystis carinii pneumonia is an extremely serious, potentially life-threatening complication that commonly affects children undergoing maintenance chemotherapy. The prophylactic use of trimethoprim-sulfamethoxazole dramatically reduces its incidence.[45]

Immunocompromised children are also at risk for serious viral infections, as disseminated varicella complicated by pneumonia, hepatitis and cerebral infection could be fatal, as well as severe measles infection. This can be explained by partial or complete loss of protective antibody titers against vaccine preventable diseases making leukemic children (especially younger ones) more susceptible to infections than general population. Antibody titers to live attenuated viral vaccines such as MMR and oral polio and recombinant hepatitis vaccine are usually the most affected by chemotherapy, when compared to those against bacterial antigens as tetanus, diphtheria and pertussis. Care must also be taken to immunize adequately susceptible household contacts of these children.[33,53,54]

Many investigators studied the safety of booster vaccine doses in these children and their effect on antibody titers.[46,49,50,55-58] Fioredda et al[59] summarized general rules concerning immunization of immunosuppressed children; and advised to avoid re-immunization before 3–6 months after the end of therapy because of possible weak or absent response. They made an exception in case of high-risk contact or high endemic risk (e.g. HBV), where immunization can be performed even during chemotherapy, keeping in

mind that the immunologic response may be weak. Vaccinations against influenza and encapsulating bacteria are recommended. Seasonal immunization against influenza is recommended during the less aggressive phases of treatment, and even within the first 6 months after the end of chemotherapy. Sung et al[60] added that the receipt of blood products can interfere with responses to live viral vaccines and a washout period before revaccination is required.

Current CDC recommendations for immunizations of cancer patients:[32]

- Immunocompromised patients should not be administered live vaccines.
- Oral polio vaccine should not be administered to any household contact of a severely immunocompromised person.
- Measles–mumps–rubella (MMR) vaccine is not contraindicated for the close contacts (including health-care providers) of immunocompromised persons and should be given to susceptible household members.
- Patients with leukemia in remission status who have not received chemotherapy for at least 3 months are not considered severely immunosuppressed and can receive live virus vaccines.
- When chemotherapy or immunosuppressive therapy is being considered vaccination ideally should precede the initiation of chemotherapy or immunosuppression by greater than or equal to 2 weeks.
- Vaccination during chemotherapy or radiation therapy should be avoided.
- Patients vaccinated while on immunosuppressive therapy or within 2 weeks before starting therapy, should be considered unimmunized and should be revaccinated at least 3 months after discontinuation of therapy.
- When exposed to a vaccine-preventable disease such as measles, severely immunocompromised children should be considered susceptible regardless of their history of vaccination.

Finally, the mechanisms responsible for immune reconstitution in leukemic patients after end of therapy are not definitively understood. It is commonly described that neutrophils usually return to normal levels within a short time, followed by NK cells, while B and T-lymphocytes levels take longer to recover. Evaluation of protective antibodies is usually performed by measuring antibodies titer in serum, but its timing is important because if it is performed too early after the end of therapy it could be negative due to the incomplete reconstitution of the immune system. In contrast, if the serum antibodies titration is done too late it might be insufficient because too much time would have gone since the original immunization.[59]

Moreover, it should be kept in mind that younger patient, especially who had not completed the primary schedule, are less protected against vaccine antigens than the older ones. Fioredda et al[59] concluded that deciding whether to re-vaccinate, give a booster shot, or do nothing at all to patients who have completed their vaccination schedules is not easy due to the lack of literature data on this topic.

Hepatitis B infection

Hepatitis B virus (HBV) infection remains a global public health problem despite the availability of an effective vaccine.[61]

Epidemiology

In the United States, the incidence of acute hepatitis B in children (<19 years) has decreased from approximately 13.8 cases per 100,000 population (10 to 19 years) in the 1980s to 3.03 and 0.34 cases per 100,000 population in 1990 and 2002, respectively. Declines have been greatest among children who were born after 1991 when recommendations for universal hepatitis B vaccination of infants were implemented.[62] By 2005, the incidence of acute hepatitis B in children < 15 years had fallen to 0.03 cases per 100,000 population.[63]

Most cases of acute hepatitis B infection in the United Stats, and in several other non-endemic countries, develop in patients from high-risk groups such as intravenous drug users, homosexual men, in those living in communities with a large proportion of immigrants from regions where HBV is endemic, and in certain groups where HBV is endemic, such as Native Americans in Alaska.[64] The majority of children with new HBV infections are immigrants, have immigrant parents, or become exposed through other household contacts.[65]

In countries where HBV is endemic, perinatal transmission remains the most important cause of chronic infection because of high rates of disease in pregnant women. Perinatal transmission also occurs in non-endemic countries, including the Untied states, mostly in children of HBV-infected mothers who do not receive appropriate HBV immunoprophylaxis at birth.[66]

Egypt is considered to be a region of intermediate prevalence for HBV infection with a reported figure of 4.5%. The most important epidemiologic factor affecting the chronic carrier rate is age of infection. The earlier in life an infection occurs, the higher the probability that this infection will result in chronic carriage; 90% of infants, 25%-50% of children 1-5 years and > 5% of adults who acquire the infection become chronic carriers. (Table 7).[67]

Screening for HBV infection should be performed by investigating the presence of HBsAg and anti-HBs. Patients who are negative for these markers should be vaccinated.[68]

Clinical manifestations

Infection with HBV is associated with characteristic changes in the serum levels of hepatitis B antigens and antibodies. These markers are sued to define different clinical states.

Acute HBV infection

Acute HBV infection in children has a variable course ranging from asymptomatic infection to fulminant hepatitis. Universal HBV vaccination has substantially reduced the frequency of fulminant hepatitis. As an example, mortality in Taiwan decreased from 5.36 to 1.71 per 100,000 during the last two decades of the 20th century. [69,70]

Clinical manifestations in children who develop symptoms are similar to those in adults. The incubation period lasts one to four months. A serum sickness-like syndrome may develop during the prodromal period, followed by constitutional symptoms, anorexia, nausea, jaundice and right-upper-quadrant discomfort. The symptoms and jaundice

generally disappear after one to three months, but some patients have prolonged fatigue even after normalization of serum aminotransferase concentrations. However, some features are seen more commonly in certain age groups: [71]

The proportion of patients progressing to chronic infection is much higher in neonates (up to 90 percent) compared with 1 to 5 percent in adults and intermediate values in young children.

* Most older children and adolescents have mild constitutional symptoms during acute HBV infection. Fulminant hepatitis is rare, but can also be seen, particularly in infants born to mothers who are HBsAg-positive and HBeAg-negative.
* Gianotti-Crosti syndrome (characterized by papular acrodermatitis of the face, extremities, and trunk accompanied by lymphadenopathy) is seen in infants and young children with acute HBV infection in whom it may be the only clinical manifestation. The syndrome is not specific to HBV infection, since it can be seen with other forms of viral infection as well.

Table (7): Epidemiology of hepatitis B virus worldwide.[67]

	High	Intermediate	Low
Carrier rate, percent	≥ 8 percent	2-7 percent	≤1percent
Geographic distribution	Southeast Asia; China; Pacific Islands; sub-Saharan Africa; Alaska(Eskimos)	Mediterranean basin; Eastern Europe; central Asia; Japan; Latin and South America; Middle East	United States and Canada, western Australia; New Zealand; Western Europe
Predominant age of infection	Perinatal and early childhood	Early childhood	Adult
Predominant mode of infection	Maternal-infant; percutaneous	Percutaneous; sexual	Sexual; percutaneous

Chronic HBV infection

The diagnosis of chronic HBV infection is based on persistence of HBsAg for more than six months; IgG anti-HBc is positive, while IgM anti-HBc is negative (Table 8).[72]

Most children with chronic HBV infection are asymptomatic and grow and develop normally. Some children note vague right upper quadrant discomfort and fatigue. Chronic HBV infection is occasionally associated with extrahepatic manifestations including polyarteritis nodosa and glomerulonephropathy; the latter is observed principally in children. HBV infection can induce both membranous nephropathy and, less often, membranoproliferative glomerulonephritis. The typical presentation is with nephritic range proteinuria. Approximately 30 to 60 percent of children with HBV-related membranous nephropathy undergo spontaneous remission, usually in association with HBeAg to anti-HBe seroconversion (i.e. conversion from HBeAg positive to negative, and from anti-HBe negative to positive). The incidence of HBV associated membranous nephropathy is falling as rates of HBV vaccination increase.[73]

Natural History of chronic infection

The natural history of chronic HBV infection in children is variable, depending upon age, mode of acquisition, and ethnicity. These differences are likely due to immune tolerance that develops when infection occurs at an early age. The exact mechanism through which immune tolerance develops are unknown.

- Children from endemic countries in whom HBV was acquired perinatally usually remain HBeAg-positive and have high levels of viral replication, although histologic injury is typically mild.[74] In an illustrative report from Egypt, approximately 90% of infants, 25%-50% of children 1-5 years and > 5% of adults who acquire the infection become chronic carriers.[75]
- By contrast, children in non-endemic countries are less likely to have aquired the disease perinataly. In this case, they frequently clear HBeAg and HBV from serum during the first two decades of life.[76] In a 29-year longitudinal study of Italian children with chronic HBV who underwent HBeAg seroconversion, 95 percent (81/85) of those without cirrhosis had inactive HBV infection at most recent follow-up, and 15 percent (13/89) cleared HBsAg.[77] Children who seroconvert spontaneously tend to have higher alanine aminotransferase (ALT) levels early in life as compared with those who do not seroconvert spontaneously.

Phases of chronic HBV infection[77]

Individuals with perinatally acquired chronic HBV infection typically enter an immune tolerant phase, from which they eventually move on to an immune active phase and hence to spontaneous clearance of the infection or to an inactive carrier state. The timing of this progression varies between individuals, and the severity of liver injury may depend on the length of the immune active phase.

*** Immune tolerant phase**

This phase is characterized by normal or mildly elevated serum aminotransferase activity (ALT <2 times the upper limit of normal) and evidence of active HBV replication (HBV DNA >20,000 IU/mL). HBsAg and HBeAg are positive.

*** Immune active (clearance) phase**

This phase is characterized by elevated serum aminotransferase activity (ALT>2 times .the upper limit of normal) and active HBV replication (HBV DNA >20,000 IU/mL). HBsAg and HBeAg are positive. Patients in this phase are more likely to clear HBeAg spontaneously or to respond to treatment than those in the immune tolerant phase.

*** Inactive, non-replicative, or latent phase**

This phase is characterized by normal levels of serum aminotransferase activity and low or undetectable levels of HBV replication. HBsAg is positive but HBeAg is negative. Patients may revert to the immune active phase at any time, so regular monitoring is necessary. These patients also remain at risk for complications of HBV such as hepatocellular carcinoma.

*** Clearance of infection**

A minority of patients who clear HBeAg also go on to clear the HBV infection, heralded by clearance of HBsAg and appearance of HBsAb. With rare exceptions, these individuals will not revert to active HBV infection, but they remain at risk for hepatocellular carcinoma due to precore mutations.

Progression to cirrhosis[76]

Cirrhosis appears to be an infrequent complication of HBV infection during childhood, although precise estimates of its incidence are uncertain. One of the largest studies done in Italy included 292 consecutive children who were HBsAg positive and had an elevated serum ALT level.[77] Cirrhosis was found in 10 patients (3 percent) at a mean age 4.0±3.3 years. However, children with cirrhosis had a higher prevalence of hepatitis D virus (HDV) infection and were more likely to have had blood transfusions suggesting that coinfection with HDV or hepatitis C virus (HCV) may have contributed to disease progression in some children. No child developed cirrhosis during follow-up (ranging from 1 to 10 years) .The risk is higher among individuals from areas endemic for HBV infection, most of whom acquire the disease perinatally, and individuals who abuse alcohol probably have accelerated disease progression.

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) in children and adults with HBV infection has been described in both Asian and Western populations.[78-82] Adults with perinatálly acquired HBV develop HCC at a rate of about 5 percent per decade.[83] The risk is related to the duration of disease, the degree of histologic injury, and the replicative state of the virus (HBV DNA levels). The risk is higher in patients who are HBeAg positive, as compared with. those who are HBsAg positive but HBeAg negative (except for those with precore mutants). The risk is increased further in the presence of cirrhosis or concomitant infection with HCV or HIV. However, HCC has been described in children who had undergone early HBeAg seroconversion, indicating that there is still a risk for HCC even after viral replication ceases. [84]

HBV genotype may influence HCC development in children differently than in young adults. In Taiwan, the majority of children with HCC and chronic HBV infection have HBV genotype B.[85] In contrast, most studies in young adults report an association between HBV genotype C and HCC.

Universal childhood vaccination has led to a major decline in the incidence of HCC in endemic countries. In Taiwan, for example, the average annual incidence in children six to nine years of age has decreased from 0.7 to 0.57 to 0.36 per 100,000 between the years 1981 to 1986, 1986 to 1990, and 1990 to 1994, respective1y.[86]

Table (8): Glossary of clinical terms used in HBV infection [83]

Definitions
***Chronic hepatitis B:**
Chronic necroinflammatory disease of the liver caused by persistent infection with hepatitis B virus. Chronic hepatitis B can be subdivided into HBeAg positive and HBeAg negative chronic hepatitis B.
*Inactive HBsAg carrier state:
Persistent HBV infection of the liver without significant, ongoing necroinflammatory disease.
*Resolved hepatitis B:
Previous HBV infection without further virological, biochemical or histological evidence of active virus infection or disease.
*Acute exacerbation or flare of hepatitis B:
Intermittent elevations of aminotransferase activity to more than 10 times the upper limit of normal and more than twice the baseline value.
*Reactivation of hepatitis B:
Reappearance of active necroinflammatory disease of the liver in a person known to have the inactive HBsAg carrier state or resolved hepatitis B.
*HBeA clearance:
Loss of HBeAg in a person who was previously HBeA positive.
*HBeAg seroconversion:
Loss of HBeAg and detection of anti-HBe antibodies.

Hepatitis B virus vaccination

Introduction

There are more than 2 billion individuals with serological evidence of hepatitis B infection worldwide. Of these, 400 million are chronic carriers and 500,000 to 1.2 million will die annually from cirrhosis and hepatocellular carcinoma. Despite advances in antiviral therapy, only a minority of patients with chronic hepatitis B will have a sustained response. Thus, primary prevention by vaccination to increase herd immunity remains the main thrust in the control of HBV infection.[87,88]

Currently available hepatitis B vaccines are extremely safe[89] and have an efficacy of >90 percent. Thus HBV infection can potentially be eradicated through global vaccination. Unfortunately, vaccination coverage is low in many developing countries because of the lack of funding and infrastructure to purchase and deliver the vaccines. Vaccination coverage is also low in many developed countries because of the misconception that vaccination is only necessary in high-risk groups in non-endemic areas. Even in countries that actively advocate universal vaccination, coverage is less than 100 percent (eg, only 87 percent in Taiwan). [90] As of 1999 in Taiwan, the vaccine coverage was >90 percent for children younger than 8 years of age but only between 80 to 86 percent among children between 8 and 15 years of age. [90] Furthermore, 5 to 10 percent of the population will not respond to currently available vaccines. Thus, public health education and infection control measures to interrupt transmission of HBV remain important.

The development of hepatitis B vaccine is considered to be one of the major achievements of modern medicine. Three different classes of hepatitis B vaccine are available based upon how they are derived (from plasma, yeast, or mammalian cells).

The first generation HBV vaccine was prepared by concentrating and purifying plasma from HBsAg carriers to produce 22 nm subviral particles, which contain HBsAg alone. Derivation from plasma has left lingering concerns regarding the potential to transmit blood-borne infections, although this vaccine has excellent efficacy and safety. [91] As a result, the plasma-derived vaccine is no longer used in most developed countries. On a global scale, it constitutes 80 percent of all vaccines produced because of the relatively low cost of production and the abundant source of HBsAg positive plasma in endemic countries.[92]

Yeast-derived recombinant HBV vaccines were first introduced in the mid 1980s. They are produced by cloning of the HBV S gene in yeast cells. These vaccines contain non-glycosylated HBV small S protein as the envelope antigen which must be released from the yeast during the manufacturing process. These vaccines do not contain, antigens of the pre-S regions.[93]

A concern related to the original versions of the recombinant vaccines is that they contained thimerosal (an organic mercurial) as a preservative. There has been no evidence of harm caused by the level of exposure that children may encounter during existing immunization schedules. Nevertheless, the potential risk of abnormal neurodevelopment prompted a joint statement from the American Academy of Pediatrics and the United States Public Health Service calling for the removal of thimerosal-containing vaccines as soon as possible, and for expeditious replacement with preservative-free vaccine.[94,95] However, the joint statement issued interim recommendations highlighting that the risk of not vaccinating children for outweighs the unknown and probably small risk (if any) of cumulative exposure to thimerosal. Two yeast-derived recombinant thimerosal-free vaccines have been developed (Recombivax HB and Engerix-B) and are widely available.[96]

The third class of vaccine is the mammalian cell-derived recombinant vaccine. Three vaccines of this class have been developed. In addition to the S antigen, one of these contain antigen from the pre-S2 region while the other two contain antigens from both the pre-S1 and pre-S2 regions. A controlled trial suggested that this class of vaccine was associated with an enhanced immunologic response compared to Engerix-B in a three-dose regimen and was equally effective in a two-dose regimen.[97] Although vaccines with pre-S antigens may be more immunogenic, they are not widely available.

A combination vaccine (Twinrix®, GlaxoSmithKline), including Engerix-B and HAVRIX (hepatitis A vaccine), is also available.[98-100] It has been approved for use in adults in the United States and Europe, and in children in some countries. Approval was based upon data from 1,551 study participants from 11 clinical trials who received Twinrix® on a zero, one and six-month schedule. An immunogenic response against hepatitis A and B was observed in 99.9 and 98.5 percent of vaccinees, respectively. An accelerated dosing schedule (with doses given at 0, 7, and 21 to 30 days, and a booster at 12 months) has also been approved. Its main potential advantage is convenience and improved compliance for those who require vaccination against both hepatitis viruses.

A candidate vaccine has been developed with a novel approach to its administration. It consists of the surface and core antigen of the hepatitis B virus administered as a nasal spray of 125 microliters per nostril at a five-dose schedule of 0, 7, 15, 30, and 60 days. A phase 1 trial reported anti-HBc seroconversion of 100 percent and development of anti-HBs antibodies in 75 percent.[101] Further studies are needed to determine the safety and efficacy of this vaccine. If the initial results are confirmed, this novel mode of vaccine administration may potentially increase vaccine use in countries where parenteral adminstraton is not readily available.

Indications

Hepatitis B vaccine is recommended for all neonates of HBsAg positive mothers, and in many countries is also recommended for neonates of HBsAg negative mothers.

Recommendations for screening the adult population have been issued by two major organizations in the United States: the United States Preventive Services Task Force (USPSTF) and the American Association for the Study of Liver Disease (AASLD).[102,103] The USPSTF provides a strong recommendation for screening women during their first prenatal visit. They recommend against screening the general asymptomatic population citing the lack of evidence that such screening improves health outcomes, and could possibly cause harm (principally from stigmatizing patients in whom screening is being performed).

The AASLD recommends screening certain high risk groups and vaccinating those who are not already immune or infected. This includes (in addition to pregnant women) persons born in hyperendemic areas, injecting drugs users, patients on dialysis, HIV infected patients, and family and household contacts of HBV-infected persons.

Neonates of HBsAg positive mothers

Vaccination of neonates of HBsAg positive mothers is the most important step toward the eradication of chronic HBV infection. Vaccination of newborns of carrier mothers is extremely cost-effective with an estimated cost per year of life saved of $164.[104] The standard regimen consists of passive and active immunization. Hepatitis B vaccine and hepatitis B immunoglobulin (HBIG) are given at the same time at two different sites within 12 hours of delivery. The neonates then proceed to two additional' doses at months 1 to 2 and months 6 to 12. This regimen has a protective efficacy of 95 percent.[105,106]

Studies in Hong Kong found that the protective efficacy of vaccine alone was significantly lower (only 75 to 80 percent).[106,107] One study from Thailand found that three doses of hepatitis B vaccine without co-administration of HBIG achieved equivalent protective rates.[108,109] These data remain to. be confirmed. It is possible that vaccine alone may be effective in countries in which most of the maternal carriers are HBeAg negative and have undetectable HBV DNA in serum. Until the general applicability of this strategy is proven, passive-active immunization should be the standard regimen for newborns of carrier mothers. A statement by the USPSTF noted that the current practice of vaccinating all infants against HBV infection and post exposure prophylaxis with hepatitis B immune globulin administered at birth to all infants of HBV infected mothers substantially reduces the risk for acquiring HBV infection.[102]

All neonates

Universal vaccination of all newborns regardless of maternal HBsAg status is necessary for global eradication of HBV infection. Experience in the United States demonstrated that vaccination programs targeted at high-risk groups had very little impact on the incidence of HBV infection.[110] Other than health care workers, the recognition and recruitment of high-risk individuals have been unrewarding. The Centers for Disease Control and Prevention (CDC) estimated that only 7 percent of high-risk individuals were vaccinated by 1988. In addition, a high proportion of chronic infection acquired during childhood occurred among children born to mothers who are not infected with hepatitis B virus.[111]

In 1991, the Global Advisory Group of the Expanded Program on Immunization recommended integration of the hepatitis B vaccine into national immunization programs, setting 1995 as a target date for countries with a HBV carrier prevalence of 8 percent or higher, and 1997 as the target date for all other countries. WHO endorsed the recommendation in May 1992, and the World Health Assembly added a disease reduction target for hepatitis B in 1994, calling for an 80 percent decrease in new HBV carrier children by 2001. As of December 2007, 171 countries have introduced hepatitis B vaccine into their national immunization program.[112] Countries that have adopted this recommendation have experienced a marked reduction in carrier rates. This has been most evident in regions with a high prevalence of chronic HBV. The reduction in carrier rates ranged from 16 percent before implementation of the program to 0 percent after implementation in Alaska, 7 percent to 0.5 percent in Samoa-New Zealand, and 12 percent to 3 percent in Micronesia-Australia. Universal vaccination of all newborns was recommended in the United States in l99l.[113]

Egypt was one of early countries to adopt HB vaccination of infants.[114] Reviewing the information about HBV carriage in Egypt, it has been reported.[115] to range from 3-11% in the general population. Egypt adopted the global strategy for control of HBV by implementation of HB vaccination through EPI .Full scale implementation started since October 1992 with coverage rate about 90%with 3 doses of recombinant vaccine were given at 2,4,6month.[116]

Dose regimen

Two yeast-derived recombinant hepatitis B vaccines have been licensed in the United States: Engerix-B and Recombivax HB. Engerix-B is formulated to contain 20 mcg HBsAg/mL while Recombivax HB contains 10 mcg HBsAg/mL.

There are three different manufacturers producing mammalian cell-derived vaccines with pre-S epitopes. Gen Hevac B (Pasteur, France) contains 20 ug/dose of vaccine, Bio-Hep-B/Sci-B-Vac (Bio-Technology General, Israel) contains 2.5 to 10 ug/dose while AG-3 (Hepagene, Hepacare)(Medeva, UK, Evans UK) contains 10 to 20 ug/dose of vaccine.[117] In most countries, the regimen for these mammalian cell-derived recombinant vaccines in similar to the yeast-derived ones; three doses at one and six months apart.

Infants born to mothers with unknown or known positive HBsAg status must receive the first dose of vaccine within l2 hours of birth, and the second and third doses at one to two months and at six months, respectively. Administering the birth dose serves as a "safety net" to prevent perinatal infection among infants born to HBsAg-positive mothers who were not identified because of erroneous maternal HBsAg testing or failure in reporting test results. In addition to providing early protection among infants at risk of perinatal infection, it is also associated with higher rates of on-time completion of the hepatitis B vaccine series.[118] Slight flexibility is permitted in the vaccination schedule for infants born to mothers with negative HBsAg status. The first dose should be administered before hospital discharge, the second dose at 1 to 2 months, and the third dose at 6 to 18 months.[118]

Vaccines should be administered intramuscularly since deposition of the vaccine into adipose tissue result in a lower seroconversion rate.[119] Thus, the deltoid is the preferred site in adults while the vastus lateralis is preferred in infants. Longer needles should be used in overweight individuals (Table 9).

What to do about a missed dose?

Longer than recommended intervals between doses do not reduce final antibody concentrations, although protection might not be attained until the recommended number of doses has been administered.[120-123] Thus, an interruption in the vaccination schedule does not require restarting the entire series of vaccination or adding extra doses.[124,125] If the vaccination series is interrupted after the first dose, the second dose should be administered as soon as possible.[110] The second and third doses should be separated by an interval of at least two months. If only the third dose is delayed, it should be administered when convenient.

Efficacy

A positive immune, response to the vaccine is defined as the development of hepatitis B surface antibody (anti-HBs) at a titer of >10 mIU/mL. Although the level of 10 mIU/mL was arbitrarily chosen, clinical studies suggest that vaccinees with anti-HBs titer above this level are protected. The significance of this titer was illustrated in a five-year follow-up study of 773 homosexual men vaccinated in 1980; the acute infection rate increased seven times when the anti-HBs titer decreased below the level of 10 mIU/mL.[126]

Using the definition of >10 mIU/mL anti-HBs as a positive response, the overall seroconversion rate is about 95 percent in healthy adults. The rate decreases with increasing age to 86 percent in the fourth decade and 47 percent in the sixth decade.[127] The response rate is slightly lower in obese individuals, smokers, and men, and significantly lower in patients with cirrhosis or chronic renal failure, organ transplant recipients, children with celiac disease, and immunosuppressed patients. In patients on chronic hemodialysis, the response rate to recombinant vaccines is 50 to 60 percent.[128] Despite the lower seroconversion rate, the risk of hepatitis B infection is 70 percent lower in the vaccinated patients when compared with non-vaccinated patients undergoing chronic hemodialysis.[129]

Duration of protection

Although anti-HBs titers decrease with time, the duration of protection is prolonged.[127,130-134] Thus, most studies suggest that routine booster injections are not required (although there have been exceptions).[135]

The long duration of protection, despite low or undetectable anti-HBs levels, is probably due to the priming of memory cells, which are capable of eliciting anamnestic response when challenged. This is supported by the rapid increases in anti-HBs titres in previously vaccinated individuals who administered booster injections.[120] A spontaneous increase in anti-HBs titer has also been observed in association with anti-HBc seroconversion, indicating a rapid immune response to natural infection.[128]

However, the durability of the anamnestic response is incompletely understood. In a study of 105 adolescents who received HBV vaccination, an anamnestic response to booster vaccination was found in only 48 percent after 15 years suggesting that immunity may wane over time.[136] In another study, 6156 high school students vaccinated with plasma-derived hepatitis B vaccine as infants were screened serologically for sustained immunity after 15 to 18 years.[137] The authors concluded that approximately 10 percent of the total population had lost their vaccine-conferred immune response. Although it is possible that some of those who failed to respond to booster vaccination may be poor responders to the initial vaccination, this decay in immune memory raises questions about the need for surveillance for immunity and booster vaccine among high risk populations. In other studies, protection appeared to extend beyond 15 years in vaccines who have a high titre anti-HBs response (>100 mIU/mL) after the initial course of vaccination.[130,137,139]

The need for booster is controversial and the practice varies from country to country. Most health authorities will agree that booster vaccination is recommended for patients on hemodialysis in whom vaccine-induced protection may persist only as long as the antibody level is above 10 mIU/mL. For these patients, the need for booster dose should be assessed annually and booster dose administered if the antibody level declines to <10 mIU/mL.[128]

Recommendations for booster vaccination have also been proposed in a European consensus statement. Countries like Netherlands, Germany, Spain, France and Belgium recommends booster dose depending on the post-vaccination anti-HBs titer. In UK, a single booster dose is recommended only five years after primary vaccination. In the US, booster dose is not recommended for adults with normal immune status.[140] Whether there may be other groups in whom booster vaccination is required is unclear (Table 10).

Hepatitis B vaccination of immunocompromised individuals

Immunodeficient patients require higher doses of vaccine and more injections (at months 0, 1, 2, and 6) to achieve an adequate immune response. Moreover, they tend to have lower peak anti-HBs levels than immunocompetent individuals do. Primary and secondary humoral responses are also slower.[141-144]

Little information is available on the robustness and duration of immunological memory in severely immunocompromised individuals. There have been reports of clinically significant HBsAg-positive infection in dialysis patients who have lost antibody protection. Thus, regular testing for anti-HBs and a booster injection when the titer falls below 10mIU/mL is advised.[142,143]

Several studies showed that immunity against HBV was significantly affected by chemotherapy. Absence of protective serum antibody titer (anti-HBs ≥10mIU/mL) was recorded in more than half of patients who were evaluated after completing their antineoplastic regimens. This finding suggests that such patients may constitute a subpopulation susceptible to HBV infection, who may benefit from revaccination with HB vaccine.[55-58,145]

Although immunosuppressive illnesses are associated with reduced immunogenicity following vaccine administration, several studies showed good response to HB vaccine in hemodialysis patients.[144-145] Similarly, Hepatitis B vaccine seemed to be immunogenic and safe when administered to leukemic children on maintenance therapy. Several studies reported fairly good results after revaccination of cancer patients.[57,144,145,147,148]

Finally, it is important to note that not only immunocompromised children are advised for re-vaccination with HB vaccine, but booster doses of vaccine are also advised to health care personnel dealing with them.[60,149]

Table (9): Recommended doses of currently licensed formulations of hepatitis B vaccine, by age group and vaccine type[141]

	Single-antigen vaccine				Combination vaccine					
	Recombivax HB		Engerix-B		Comvax*		Pediarix		Twinrix	
Age group	Dose, µ§	Volume, mL	Dose, µ	Volume, mL	Dose, µ	Volume, mL	Dose, µ¬	Volume, mL	Dose, µ	Volume, mL
Infants (<1yr)	5	0.5	10	0.5	5	0.5	10ˇ	0.5	NA§	NA
Children (1-10yrs)	5	0.5	10	0.5	5*	0.5	10ˇ	0.5	NA	NA
Adolescents										
11-15 yrs	10¥	1.0	NA	NA	NA	NA	NA	NA	NA	NA
11-19 yrs	5	0.5	10	0.5	NA	NA	NA	NA	NA	NA
Adults (≥20 yrs)	10	1.0	20	1.0	NA	NA	NA	NA	20Δ	NA
Hemodialysis patients and other immunocompromsied persons										
<20 yrs	5	0.5	10	0.5	NA	NA	NA	NA	NA	NA
<20 yrs	40**	1.0	40″		NA	NA	NA	NA	NA	NA

* Combined hepatitis B- Haemophilus influenzae type b conjugate vaccine. This vaccine cannot be administered at birth, before age 6 weeks, or after age 5 years.

ˇCombined hepatitis B-diphtheria, tetanus, and acellular pertussis-inactivated poliovirus vaccine. This vaccine cannot be administered at birth, before age 6 weeks, or at age ≥7 years.

ΔCombined hepatitis A and hepatitis B vaccine. This vaccine is recommended for persons aged ≥1 8 years who are at increased risk for both hepatitis B virus and hepatitis A virus infections.

¬Recombinant hepatitis B surface antigen protein dose. § NA: Not applicable.

§NA:Not applicable

¥Adult formulation administered on a 2-dose schedule.

‡Higher doses might be more immunogenic, but no specific recommendations have been made.

** Dialysis formulation administered on a 3-dose schedule at age 0, 1, and 6 months. ″Two 1 .0-mL doses administered at one site, on a 4-dose schedule at age 0, 1, 2, and 6 months.

Table (10): European Consensus Group recommendations for hepatitis B booster vaccination.[140]

Children and adolescents
To date no evidence supports the use of boosters in children or adolescents. It is important to ensure that the full three-dose primary course is given.*
Healthcare workers
If post-vaccination antibody testing suggests that adequate immunological priming has not been achieved, the following course of action is recommended:
1- Screen for markers of present or past infection (HB sAg, anti-HBc)
2- Administer an additional dose of vaccine
3- Repeat anti-HBs measurement with a different assay
4- Consider passive immunization with HB immunoglobulin after exposure
Booster vaccination is not recommended for those who achieve an adequate response after primary vaccination.
Intravenous drug users
Testing should be considered only if the user has reduced immunocompetence. Currently there is no evidence to support booster vaccinations
High-risk sexual behavior
Public health services should try to identify persons with high-risk sexual behavior and ensure that they receive primary vaccination but boosters are unnecessary.
Immunocompromised patients
There is some justification for using boosters to maintain the anti-HBs levels above 10 mIU/mL in immunocompromisd individuals as recommended for dialysis patients by the US Advisory Committee on immunization Practices. Additional or double doses are recommended for non responders. Attempts should be made to administer the vaccine when the patient's immune system is likely to be functioning maximally (eg, before initiating hemodialysis). Post-vaccination testing every 6 to 12 months is advisable
Residents of mental institutions
No booster vaccinations are recommended. However, staff who are in close contact with residents should receive primary vaccination.
Close contacts of HBsAg carriers/immigrants
Close contacts of HBsAg carriers should receive primary vaccination in the absence of serologic evidence of HBV infections. No further boosters are necessary.

- A two-dose regimen has been approved for Recombivax in adolescents.