1995 to 2006 ranged between 1.49 and 1.01/100,000 population/year [6].

Viruses are etiologically associated with significant types of human leukaemia and lymphomas [7]. The Epstein-Barr virus (EBV) plays an important role and individuals with a history of infectious mononucleosis have an increased incidence of Hodgkin's lymphoma [8]. Approximately 30% to 40% of patients with Hodgkin lymphoma (HL) in the Western world and in some developing regions carry the EBV in the malignant Hodgkin Reed Sternberg (HRS) cells [9].

EBV infection is an early event in the development of HL as the viral genomes are found in a monoclonal form, indicating that infection of tumor cells has occurred before their clonal expansion [9]. Clonal viral genomes are found in the Hodgkin Reed-Sternberg cells (HRS). The latent infection results in expression of the viral oncogenes LMP-1 and LMP-2A. LMP-1 is the major transforming protein of EBV. It is a member of the tumor necrosis factor receptor (TNFR) superfamily and most closely resembles CD40. However, in contrast to CD40, LMP-1 signaling is constitutively active and requires no ligand. LMP-1 upregulates cellular Bcl-2 and other proteins that inhibit apoptosis and also stimulates cytokine production (interleukin IL-6 and IL-8) [10,11].

B cell lymphoma-2 (Bcl-2) family proteins are key regulators of the apoptotic process. Bcl-2 blocks the induction of apoptosis by inhibiting the activation of pro-apoptotic family members such as BAX and preventing mitochondrial membrane depolarization [12]. Dysregulation of Bcl-2 expression, which results in abnormal cell growth, certainly contributes to the development of some tumors [13]. Over expression of Bcl-2 may result in accumulation of cells in the G0 phase of cell cycle division [14]; causing resistance to chemotherapeutic drugs and radiation therapy, while decreasing Bcl-2 expression may promote apoptotic responses to anticancer drugs [12,15]. Consequently, Bcl-2 has become a very attractive target for the design of new anticancer drugs [16].

The study intended to evaluate the immunohistochemical expression of the Latent Membrane Protein-1 of Epstein - Barr virus and the anti-apoptotic protein Bcl-2 in Classical Hodgkin Lymphoma using a specified automated cellular image analysis system (Digimizer software analysis) then correlates their expression with clinicopathological parameters including: age of the patients and histological subtypes of the disease and to find if there is any relation between LMP-1 and Bcl-2 in classical Hodgkin's lymphoma.

Methods

This retrospective study was conducted on fifty paraffin-embedded blocks of lymph nodes biopsies from patients diagnosed as Classical Hodgkin's Lymphoma. The cases were selected from archive files of the Department of Pathology of the Teaching Laboratories, Specialized Surgical Hospital in Baghdad Medical City and Al-Kadhimiya Teaching Hospital from November 2010 to June 2011.The control group consist of 20 age matched subjects having reactive lymph nodes biopsies which were obtained from Al-Kadhimiya Teaching Hospital Laboratories. Clinicopathological parameters including the age and histological subtypes of the tumor were obtained from the available histopathological reports. Ethical approval for the use of all specimens was obtained.

For each case, three representative sections were prepared. One section stained with Hematoxylin and Eosin and the histopathological diagnosis was revised by a pathologist, while other two sections were stained immunohistochemically for LMP-1 and Bcl-2 with horseradish peroxidase (HRP)-labelled-streptavidin-biotin method. The work was done at Al-Kadhimiya Teaching Hospital Laboratories.

This technique basically uses an unlabeled primary antibody, which was mouse monoclonal antibody purchased from DAKO (code no. of the kits were IS 753 for LMP-1 and M 0887 for Bcl-2), it binds to its corresponding antigen, followed by a