Discussion
5.1 FLOWCYTOMETRIC IMMUNOPHENOTYPING OF AL:

In most laboratories in our country, diagnosis of acute leukemia (AL) is based on cytomorphology and cytochemistry (French-American-British (FAB) classification). FAB criteria broadly divide AL into AML and ALL. In the current study, of the 242 cases of AL, 46.6% (113/242) were diagnosed as AML and 53% (128/242) as ALL using FAB criteria while 0.4% (1/242) diagnosed as possible MLAL. In recent years, immunophenotyping has been increasingly used for the diagnosis and classification of acute leukemias using flowcytometry or immunocytochemistry techniques. At present, flowcytometry is the preferred method for immunophenotyping (Chianese et al., 2002; Huh et al., 2000). The goal of immunophenotyping is the identification and phenotypic characterization of blast cells. It has been reported that the concordance between experienced observers in the classification of acute leukemia increases from about 70% to >90% when morphologic criteria are supplemented with cytochemical and immunophenotypic data (Kinney et al., 1998). In the present study, FCM immunophenotyping was carried out for the confirmation of AL cases. Of the 242 AL cases, 46% (112/242) were diagnosed as AML, 51% (123/242) as ALL that included both B-ALL & T-ALL [35.5% (86/242) and 15.3% (37/242) respectively] and 3% (7/242) were diagnosed as MLAL. In a study from Tata Memorial Hospital (Mumbai), Ghosh et al., (2003) has reported that AML constitutes ~40% of acute leukemia cases, which is similar to present study (46%).

In AML, a higher frequency of M2 FAB subtypes in both childhood and adult AML has been reported in India (Advani et al., 1983; Advani et al., 1990; Ghosh et al., 2003). In the present study also, M2 was found to be the most common FAB subtypes followed by M5 in both children as well as adults. Overall, of the total AML cases, M2 (49.5%) and M5 (21%) were the most common FAB subtypes followed by M4 (12%), M1 (8%), M3 (4.5%), M0 (2.7%) and M6 (1.8%). These results were in parallel to other Indian study published by Ghosh et al., (2003) who also reported that M2 (34%) and M5 (20%) were the common subtypes. These results suggest that the frequency of AML-M2 subtype in India is higher than the frequency of 27-29% reported in the literature from other countries (Weinstein 1999; Miller et al., 2000; Jaffe et al., 2001). Incidence of M3 in the current study was 4.5%, which is similar to
the frequency of 5.4% reported by Ghosh et al., (2003). The proportion of M4 in the present study was lower (12%) compared to that reported in the literature (16-40%) (Roberts et al., 1992; Weinstein 1999; Miller et al., 2000; Jaffe et al., 2001). From India, Ghosh et al., (2003) has also reported low frequency of M4 (3.5%). In the current study, M6 was found to be the least common FAB subtypes (1.8%) while M7 was not observed as it is rare disease moiety. Ghosh et al., (2003) has also reported that M6 (1.6%) was the least common subtypes; they have also not found any case of M7 in their study.

In ALL, difference in the incidence of the morphological types in children and adults has been reported in the literature. L1 subtype is more common in children (74%) while L2 is found more common in adults (66%) (Bennett et al., 1981). These results are in contrast to present study where such difference was not seen in B-ALL i.e. in children, prevalence of L1 and L2 was 52% and 48% while in adults, prevalence of L1 and L2 was 50% each. Interestingly, in adult T-ALL, it was seen that prevalence of L1 (58%) was higher compared to L2 (33%) and L3 (8.3%). However, no difference was seen in childhood T-ALL for L1 (52%) and L2 (48%).

A FAB criterion is widely used but this classification has some limitations, as they are difficult to interpret in several cases (Bennett et al., 1994). Accurate characterization of leukemic cells using immunophenotyping has been shown to be useful in these difficult cases. The present study also revealed discrepant results in 13 FAB-diagnosed cases by immunophenotypic analysis using flowcytometric technique. Of these 13 cases, 4 cases of FAB-diagnosed AML were diagnosed as B-ALL, 3 cases of FAB-diagnosed ALL as AML and 6 cases of FAB-diagnosed ALL as MLAL. In other FAB diagnosed AL cases, AML was confirmed in 96% (109/113) cases, ALL was confirmed in 93% (119/128) cases while one possible case of MLAL was also confirmed as MLAL following FCM immunophenotyping.

It is well established that the most AL in children are of lymphoid (ALL) type whereas in adults they are typically myeloid (AML) in origin. In general the prevalence is higher among males compared to females (Weinstein 1999; Miller et al., 2000; Tyagi et al., 2006; Delhi cancer registry, 2007). Similar pattern was found in the current study. Of 112 AML patients, majority of the patients were adults (65%) while of the 86 B-ALL and 37 T-ALL patients, majority of the patients were
children (64% and 68% respectively). In the present study, males were more affected than females. Majority of the patients in AML were males (67%) and having a male to female ratio of 2:1. Similarly in B-ALL and T-ALL, majority of the patients were males (76.7% and 65% respectively) and having a male to female ratio of 3.3 and 1.8 respectively. These findings were in parallel to other published studies from abroad as well as our country. From India (Mumbai), Ghosh et al., (2003) has reported that majority of AML patients in their study were adults (76%) while Amare et al., (1999) and Agarwal et al., (2001) have reported that majority of ALL patients were children (68% and 64% respectively). Khawaja et al., (2005) and Pui et al., (1998) have also reported that majority of the ALL patients in their study were children i.e. 61% and 67% respectively.

In ALL, frequency of B-ALL was found to be higher (70%, 86/123) compared to T-ALL (30%, 37/123). In childhood and adult ALL, the frequency of B-ALL was also higher than T-ALL. The frequency of B-ALL vs. T-ALL in childhood ALL and adult ALL was 69% vs 31% and 72 vs. 28% respectively. The findings were in similar to other studies. Advani et al., (1999) has reported the frequency of B-ALL in 69% cases of ALL. The high frequency of B-ALL in both children and adults is also in accordance with the previous reports. Kamat et al., (1985) and Taskov et al., (1995) have reported similar frequency of B-ALL i.e. 67% and 66% respectively in childhood ALL. However, Agarwal et al., (2001) and Bajel et al., (2008) have reported high frequency of B-ALL in upto 80% and 92% cases of children and adults respectively. Chen et al., (2004) has also reported high frequency of B-ALL in children (76%). The frequency of T-ALL in our study was 31% and 28 % in children and adults respectively. Similar high prevalence of T-ALL in India has been found in previous studies (Kamat et al., 1985; Bhargava et al., 1988; Padmanjali, et al., 2002). Taskov et al., (1995) have also observed 28% cases of T-ALL in children. However, Agarwal et al., (2001) and Bajel et al., (2008) have reported low frequency of T-ALL in children i.e. 17% and 22% respectively. Agarwal et al., (2001) has not found any T-ALL cases in adults. Chen et al., (2004) and Khawaja et al., (2005) have also reported low frequency of T-ALL i.e. 24% (in children) and 17% (14% in children, 22% in adults) respectively. Very high proportions of T-ALL have also been reported in Egypt (50%, Kamel et al., 1989)
and from South India (44%, Rajalekshmy et al., 1997). Padmanjali, et al., (2002) has reported that the frequency of T-ALL (31%) in India was much higher in comparison to other studies from Western countries (about 15%). However, in a study on large sample size (n=739) by Thiel et al., (1989), the frequency of adult T-ALL has been reported in 26% cases which is more close to present study i.e. 28%. This suggests that the lower prevalence of T-ALL in some literature from Western countries could be because of smaller sample size.

Immunophenotyping of leukemias has been possible since the 1970s, and at present, the diagnosis of ALL depends on immunophenotyping. Since the surface marker characteristics improve the diagnostic specificity the present consensus is that diagnosis of leukemias should be based on morphology, cytochemistry and immunophenotyping. In order to establish the lineage of blast cells, immunophenotypic panel must include antigens with high sensitivity that is fully present in a certain lineage (e.g. CD7 in T-cells and CD19 in B-cells) together with more specific markers (e.g. CD3 for T-cells, or CD33 and MPO for AML). Additional progenitor cell markers such as CD34 are used to confirm the immaturity of the pathologic cells. In the current study, specific phenotypic characterization of AL blast cells was performed using lineage associated markers such as CD19 for B-cells, CD7 for T-cells and CD33 and MPO for myeloid cells.

Expression of lineage specific CD markers in AML: CD33 and MPO were expressed in ~90% cases of AML. CD33 was detected in 91% and 88% cases of children and adults while MPO was detected in 85% and 87% cases of children and adults. These findings were similar to other published study from India as well as abroad. Reading et al., (1993) Legrand et al., (2000) and Chen et al., (2005) have reported expression of CD33 in 91% cases. However, Ghosh et al., (2003) has detected CD33 in 100% cases. Reading et al., (1993) Legrand et al., (2000) and Chen et al., (2005) have reported expression of MPO in ~70% cases. Chen et al., (2005) have reported that sensitivity of CD33 (91%) is more than MPO (69%) while the specificity of MPO (100%) is higher than CD33 (62%). These findings indicate that both the marker i.e. CD33 and MPO are important and should be included in the immunophenotypic panel to confirm the diagnosis of AML.
Expression of lineage specific CD markers in B-ALL: B-lineage ALL, especially, has been classified in different ways by various study groups. In the EGIL (European group for the immunological characterization of leukemias) classification (Bene et al., 1995), at least two of the three markers (CD19, CD79a, CD22) are required to be positive for the B-lineage, which is furthermore divided into four subgroups in order of maturation. Pro-B ALL, common ALL (CD10+), pre-B ALL (cIgM+) and mature B-ALL (cytoplasmic or surface kappa or lambda). Pro-B ALL is also called pre-pre ALL in some classifications (Rothe et al., 1996). The term B-cell precursor ALL is commonly used for the B-lineage without surface immunoglobulin expression (Margolin et al., 1997). Pui et al., (1993(a)) call this same immunophenotype “early pre-B ALL”. In the current study, B-ALL cases were categorized into pro-B-ALL and common ALL as the panel used in the study included only CD 19 and CD10. CD19 is stably expressed on all stages of B lineage differentiation. In the present study, all patients showed positivity for CD19 in both children and adults. Chen et al, (2004) has also reported CD19 expression in ~99% cases of childhood B-ALL. Expression of CD 10 was observed in 94% and 97% cases of children and adults. In other words, 3% to 6% cases were negative for CD10. These cases i.e. CD19+/CD10- were classified or diagnosed as pro-B-ALL. Consolini et al, (1998) and Gleissner et al, (2005) have also reported similar frequency of pro-B-ALL (3-4%) in their study. These findings indicate that two markers i.e. CD19 and CD10 may suffice to diagnose pro-B-ALL (CD19+/CD10-) and common B-ALL (CD19+/CD10+).

Expression of lineage specific CD markers in T-ALL: CD7 (100%) was the most commonly detected CD marker followed by CD5 and CD3 in both children and adults. CD5 was detected in 83% and 90% cases children and adults while CD3 was detected in 78% and 67% cases of children and adults. Similar to present study, Chen et al., (2005) has also earlier reported that the sensitivity of CD7 (100%) was higher than CD3 (80%). Although, they have reported that the specificity of CD3 was higher (98%) than CD7 (78%). This finding was also supported by Kita et al., (1993) who have found expression of CD7 in about 20% AML patients. CD10 was less commonly seen in children (27%) compared to adults (55%). Consolini et al., (1998) has also reported lower expression of CD10 (18%) in childhood T-ALL. Overall,
these findings suggest that CD3 or preferably CD5 should be included with CD7 in the panel for immunophenotyping in order to differentiate T-ALL from B-ALL.

Expression of CD34 in AML: CD34 antigen expression has earlier been reported in 25% to 64% samples with AML. Basso et al., (2001) has discussed a number of clinical and methodological factors for the heterogeneity reported in the incidence of CD34+ AML and its association with variable survival rates. In the current study CD34 was detected in 73% and 63% cases of children and adults respectively. Chang et al., (2004) and Legrand et al., (2000) have reported CD34 expression in 65-68% in adults. More recently, Liu et al., (2007) has reported CD34 expression in ~60% cases of AML.

Several earlier reports have shown an association between CD34+ AML and lower response rate following induction therapy. Furthermore, the relapse rate has been reported to be higher in AML showing CD34 positivity compared to CD34- group. Thus, confirming the negative influence of this molecule on the clinical outcome (Vaughan et al., 1988; Borowitz et al., 1989; Campos et al., 1990; Geller et al., 1990; Lee et al., 1992; Thomas et al., 1992; Boekhorst et al., 1993; Lanza et al., 1994; Dalal et al., 1997; Raspadori et al., 1997). Contrary to this, however, there are reports in which no significant differences between CD34+ and CD34- AML patients has been reported for the outcome (Kuerbitz et al., 1992; Ciolli et al., 1993; Del Poeta et al., 1994; Lamy et al., 1994; Sperling et al., 1995; Arslan et al., 1996; Porwit-MacDonald et al., 1996; Kyoda et al., 1998). In the present study, no difference was found in the response rate of CD34+ and CD34- adult AML cases (~70% in both the group). However, in children, a trend was seen towards a lower remission rate in CD34+ (60%) cases compared to CD34- (100%) cases (p=0.4). In adults, analysis of prognostic factors revealed that CD34+ was associated with favourable Hb level (i.e. <10%) (p=0.04), while in children, it was associated with MPO positivity (p=0.03). Overall, results suggest that CD34 expression on AML blasts does not play a prognostic role in adults; however, it could influence the clinical outcome in children.

Expression of CD34 in B-ALL: Expression of CD34 was more commonly seen in adults (60%) as compared to children (35%) (p=0.07). The mean percentage positivity of CD34 was also significantly higher in adults (63 ± 20) than children (48
Similar finding in adult cases have also been reported by Cascavilla et al., (1997) and Mi et al., (1999) who found CD34 expression in 62%-76% cases respectively. However, in children, Cascavilla et al., (1997) has reported higher incidence of CD34 (73% cases) which is in contrast to the present study. The clinical significance of CD34 expression has been analysed in several studies in both adult and childhood ALL. Several studies in childhood B-ALL have reported a strong association between CD34 expression and favorable prognosis (Borowitz et al., 1990; Pui et al., 1993(b); Cascavilla et al., 1997). However, other studies have not confirmed this association and CD34 expression has been shown to be associated with major adverse prognostic factors (Sperling et al., 1995; Vanhaeke et al., 1995; Cascavilla et al., 1997). In a study by Vanhaeke et al., (1995), the outcome of CD34+ children with B-ALL was no better than the CD34- children. In the current study, CD34 positivity was associated with higher response rate in children (86% vs. 77%) and lower response rate in adults (83% vs. 100%) however, the difference was statistically not significant. In current study, in adults, LAP was more common in CD34+ (53%) than CD34- (20%) cases (0.2) while in children, high WBC (>50,000) was more common in CD34+ (53%) than CD34- (32%) cases (0.2). Overall, these results suggest that expression of the CD34 antigen is a favourable prognostic factor in childhood B-ALL and unfavorable prognostic factor in adult B-ALL.

Expression of CD34 in T-ALL: In the current study, no difference seen in the expression of CD34 between adults (22%) and children (21%) (p=1.0). In contrast to this, Cascavilla et al., (1997) has earlier reported higher expression of CD34 in both adult (55%) and children (40%) while Mi et al., (1999) has reported lower expression of CD34 expression in adults (11%). Pituch-Noworolska et al., (2001) has observed CD34 expression in 28% cases with childhood T-ALL. In current study, childhood T-ALL samples that were CD34+ had higher response rate as compared to CD34- cases (100% vs. 83%, p=1.0) although the difference was not significant, while in adults, there was a trend to a lower response rate of CD34+ cases as compared to CD34- cases (0% vs. 100%, p=0.2). Vitale et al., (2006) has also reported lower response rate in CD34+ adult T-ALL. CD34 expression has been shown to be associated with several adverse prognostic factors (Sperling et al., 1995; Cascavilla
The present study showed that all the CD34+ cases had OM and high WBC count (>50,000) versus CD34- cases i.e. (OM- 100% vs. 73%, (p=1.0), WBC-100% vs. 40%, (p=0.08) in children and OM- 100% vs. 57 %, (p=0.5), WBC- 100% vs. 43%, (p=0.4) in adults). It was also observed that in adults all the CD34+ cases (100%) showed CD10 positivity as compared to CD34- cases (43%)(p=0.4). However, in children no such difference was seen between CD34+ vs. CD34- cases (25% vs. 33%, p=1.0). As in B-ALL, our results suggest that expression of the CD34 antigen is a favourable prognostic factor in childhood T-ALL and unfavorable prognostic factor in adult T-ALL.

Clinical, hematological and cytochemical findings in AL patients:

AML: Clinopathological features showed that Organomegaly (OM) was more common than lymphadenopathy (LAP) in both childhood and adult AML. OM was observed in more than 50% of patients while LAP was seen in less than 40% of patients. LAP was more common in children than adults (38% vs. 16%, p=<0.01). No difference was found in the mean Plt count and WBC count of children and adult ALL. However it was found that Mean Hb% was significantly lower in children than adult AML (5.8 vs. 6.7%, p=0.05). MPO as well as AR was seen in majority of the patients (~90% and ~70% respectively), it was expected as these are the important markers for diagnosing AML.

ALL: Clinopathological features showed that the majority of the B and T-ALL patients had OM (~70-80%) and LAP (~40-70%). However, LAP was more often associated with T-ALL (~70% in both children and adults) than B-ALL (~50% in children and ~40% in adults). These findings were similar to Advani et al., (1999) who observed that LAP and OM was present in about 80% cases of ALL. LAP occurs more frequently in patients with T-ALL and reported to be of prognostic significance (Shuster et al., 1990). Advani et al., (1999) has also observed that LAP was more common in T-ALL (~90%) than B-ALL (~75%). No difference was found in the mean Plt and WBC count of children and adult ALL. However, mean Hb% was significantly lower in children than adult B-ALL (6.0 vs. 7.5%, p=0.02) but such difference was not observed in T-ALL. The mean Hb% of childhood B-ALL was also found to be significantly lower compared to childhood T-ALL (6.0 vs. 7.7%, p=0.01). PAS positivity was more common in B-ALL (~80%) than T-ALL (~60%).
Chapter-V

5.2 ABERRANT ANTIGEN EXPRESSION IN AL:

Occurrence of aberrant phenotype in AL and its prognostic significance differs considerably in independent studies in children as well as in adults. The aim of the current study was to analyse the frequency of aberrant phenotype and to correlate aberrant phenotype with known adverse prognostic factors in childhood and adult acute leukemia. In the present study, a cut-off value of >20% for aberrant marker was used to define aberrant phenotypes.

In the present study, Ly+ AML phenotype was found in 49% of all AML cases, which is comparable to other studies that reports 40% to 54% of aberrant phenotype in all AML cases (Reading et al., 1993; Macedo et al., 1995). In children, Ly+ AML phenotype was found in 59% cases of AML which is similar to 61% of Kuerbitz et al. (1992). However, there are studies that report low frequency of aberrant phenotype i.e. from 24% to 35% (Shen et al., 2003; Smith et al., 1992; Kawai et al., 1995). In adults, Ly+ AML phenotype was found in 45% cases of AML, which is in parallel to other reports (Khalidi et al., 1998; Legrand et al., 2000; Bahia et al., 2001; Cruse et al., 2005; El-Sissy et al., 2006).

Among the lymphoid associated antigen, expression of CD19 in our study was more common than CD7 in both adult and childhood AML that is in contrast to other studies. Expression of CD7 (16% to 37%) has been reported the more common lymphoid associated antigen than CD19 (10% to 16%) (Khalidi et al., 1998; Reading et al., 1993; Legrand et al., 2000). Ball et al. (1991) have also reported low expression of CD19 (14%) in adults. In the current study, CD19 and CD7 were expressed in 32% and 15% cases of adults AML respectively. Aberrant expression of CD7 (15%) in our study was comparable to other studies that report CD7 expression from 12% to 17% cases of adult AML (Zhu et al., 2002, Shen et al., 2003; El-Sissy AH et al., 2006). However, in the literature, expression of CD7 has been reported from 9% to >30% cases of AML (Lo Coco et al., 1989; Zutter et al., 1990; Jensen et al., 1991; Kita et al., 1993; Del Poeta et al., 1994; Saxena et al., 1998; Cruse et al., 2005). In childhood AML, the most commonly expressed lymphoid antigen was CD19 (52%) followed by CD7 (14%). Aberrant expression of CD7 (14%) in childhood AML was comparable to 17% of Kawai et al. (1995). In the present study, CD3 and CD5 was found to be positive in one case of adult AML, literature also
reports low expression of CD3 and CD5 in AML (Kuerbitz et al., 1992; Smith et al., 1992; Legrand et al., 2000; Lewis et al., 2007).

Among the FAB subtypes of AML, aberrant expression of CD19 and CD7 was more commonly found in M5 subtypes. However, Kita et al., (1992) have reported expression of CD19 in 78% cases of M2 subtypes having t(8;21) translocation. Ball et al. (1991) have reported expression of lymphoid antigens more common in M3 (28%) followed by M1 (25%), M4 (14%) and M5 (13%). In the present study, expression of CD7 in childhood AML was more commonly seen in M5 subtype (43%) than M2 subtype (6%) (p=0.05).

In ALL, myeloid associated antigen expression has been reported from 10% to 47% cases of ALL (Drexler et al., 1991; Boldt et al., 1994; Preti et al., 1995; Shen et al., 2003). Sobol et al., (1987) have reported My+ phenotype in 33% cases of adults. In our study, My+ phenotype (CD33+) was found in 39% and 33% cases of adult B-ALL and T-ALL respectively which is comparable to a recent study, that reports expression of CD13 and/or CD33 in 38% and 24% cases of adult B-ALL and T-ALL respectively (Vitale et al., 2007). However, Thalhammer-Scherrer et al., (2002) have reported low expression of CD33 in B-ALL (3%) and T-ALL (7%) cases of adults. In children, My+ phenotype has been reported in 36% and 30% cases of B-ALL and T-ALL respectively (Shen et al., 2003). In our study, My+ phenotype was found in 23% cases of childhood B-ALL which is comparable to the recent studies that report aberrant phenotype in 23% to 27% cases of childhood B-ALL (Suggs et al., 2007; Ng et al., 2007). Interestingly we have not observed expression of CD33 in any of the 24 cases of childhood T-ALL. Suggs et al., (2007) have also reported no expression of CD33 in childhood T-ALL (in less than 13 year). However, Uckun et al., (1997) have found expression of CD33 in 19% cases of childhood T-ALL.

Among the FAB subtypes of childhood B-ALL no difference was found in the frequency of My+ phenotype in L1 (22%) and L2 (23%) subtypes while in adult B-ALL, frequency of My+ phenotype was found to be higher in L1 (44%) compared to L2 (33%) subtypes. However, Pui et al. (1991(b)) and Wiersma et al. (1991) have observed My+ phenotypes more common in L2 subtypes than L1.
Association of aberrant phenotype with adverse prognostic factors and clinical outcome is still the controversial issue. Aberrant phenotypes (Ly+ AML) have been shown to be associated with the poor prognosis, however, other studies report favorable prognosis \(\text{[Smith et al., 1983; Cross et al., 1988; Ball et al., 1991].} \)

\textit{Drexler et al., (1993)} have reported no difference in the treatment outcome and clinical features of aberrant and normal phenotype. In our study, we have also found no difference in the treatment outcome of Ly+ and Ly- phenotype. The rate of CR in adults was 60% and 57% in Ly+ AML and Ly- AML respectively while the rate of CR in children was 78% and 100% in Ly+ AML and Ly- AML respectively. Recently, \textit{Zheng et al., (2008)} have also showed that the presence of lymphoid-associated cell surface antigens in childhood AML posses no prognostic value. \textit{Kawai et al., (1995)} have reported no significant difference in the clinical and hematological findings of Ly+ and Ly- AML phenotypes. We also found no significant difference between the clinical and hematological findings of Ly+ and Ly- AML phenotype of adults as well as of children. However, absence of auer rods (-), a poor prognostic factor, was significantly found to be associated with Ly+ AML of children \( (p=0.01) \). The significance of this finding in terms of clinical outcome could not be evaluated as the sample size of Ly-AML group was small. However, \textit{Creutzig et al., (1995)} have reported good outcome in children showing auer rods in their blasts.

Association of aberrant phenotypes with prognostic factors and clinical outcome is also controversial in childhood ALL \(\text{[Wiersma et al., 1991; Reiter et al., 1992; Pui et al., 1993(a); Uckun et al., 1997; Ng et al., 2000]}\) and adult ALL \(\text{(Drexler et al., 1991; Yenerel et al., 2002).} \) In adults ALL, favorable outcome has been reported in aberrant phenotype \(\text{(Yenerel et al., 2002).} \) \textit{Sobol et al. (1987)} have reported no significant differences in presenting clinical features of adult My+ and My- ALL. Moreover, the authors have reported poor response in aberrant phenotype of B-ALL but not in T-ALL. In our study, both adult and childhood B-ALL patients showed no significant difference in the clinical outcome of My+ B-ALL and My- B-ALL phenotype. No significant difference was also found between adverse prognostic factors of adult My+ ALL and My- ALL (B-cell and T-cell). Recently, \textit{Vitale et al., (2007)} have also showed no difference between adult My+ and My-
phenotype in terms of presenting clinical, hematological and biological features and clinical outcome.

Expression of CD34 in AML is admittedly regarded as a negative prognostic factor. However, in ALL, CD34 is considered as positive prognostic factor that is associated with known favorable variables in children but not in adults (Borowitz et al., 1990; Pui et al., 1993(b); Cascavilla et al., 1997). In the current study, no significant difference was found between aberrant and normal phenotypes for the CD34 marker in both adults and children. However, Kawai et al. (1995) have reported that CD34 was more associated with Ly+ phenotype (91%) than Ly- phenotype (31%) of childhood AML. Overall, these results indicate that expression of CD34 is not associated with aberrant phenotype. However, CD34 was more commonly seen in My+ phenotype of adult B-ALL (64%) than childhood B-ALL (25%) (p=0.09).

5.3 EVALUATION OF CELL VIABILITY AND APOPTOSIS AND ITS CORRELATION WITH CLINICAL OUTCOME

An indicator that may possibly be directly related to the prognosis and recurrence of acute leukemia after induction therapy is the link between cell survival, cell proliferation and cell death with clinical drug resistance. It has been proposed that increased leukemic cell survival is related to increased drug resistance leading to failed induction chemotherapy (Ong et al., 2000; Srinivas et al., 2000; Pallis et al., 2003). Spontaneous apoptosis of blast cells after incubating them for different time period have earlier been correlated with the clinical response after induction chemotherapy (Norgaard et al., 1999). However there is scanty information on the number of circulating apoptotic blast cells in the peripheral blood samples of patients with acute leukemia. Moreover measuring spontaneous apoptosis after incubation is dependent on availability of facilities for short-term culture of blast cells. This prompted us to evaluate if clinical response of the patients could be correlated with the number of circulating apoptotic blast cells in the peripheral blood of these patients. For this, cell viability of blast cells was done immediately after separating them from freshly drawn blood samples using 7-AAD and Annexin V + PI.

It has been reported that spontaneous proliferation as well as spontaneous apoptosis in the leukemic cell lines after 24 hour of serum-free culture parallel their
drug sensitivity: K562 cells, being the least sensitive display the least apoptosis at 24 hour (2.5%) and REH cells, the most sensitive the maximum apoptosis (24.4%) (Lowenberg et al., 1993; Smith et al., 1998). Li et al., (1994) have reported 0.1% to 16 % apoptotic cell (prior to chemotherapy) in leukemic patients but they have not evaluated its prognostic significance. In vitro fresh leukemic blast cells of patients with AML have also been found to have a significant correlation between blast cell survival and proliferation with drug resistance and a poor response to chemotherapy. There was a 19.5% apoptotic AML cell after 24 h of serum-free culture in patients who entered a CR compared with 4.2% apoptotic AML cells in patients who did not achieve a CR (Norgaard et al., 1996; Smith et al., 1998; Norgaard et al., 1999).

Several factors that can affect the apoptosis in acute leukemia have been described. Altered expression of members of the Bcl-2 family such as higher Bcl-2, Bcl-xL and lower Bax expression levels have been reported in AML cells resistant to apoptosis and thus to chemotherapy. It has also been shown that CD34 positive fraction is more resistant to apoptosis than CD34 negative fraction. In multidrug resistant protein (MRP)-positive patients, CD34 positivity and P-glycoprotein activity is related with a lower rate of CR, a high rate of leukemic relapse and resistance to spontaneous apoptosis (Laupeze et al., 2002; Pallis et al., 2003; Van et al., 2003). A positive correlation between apoptosis, mutant p53gene and Bax expression and a negative correlation to the expression of Bcl-2 protein has been reported in pediatric ALL patients (Srinivas et al., 2000). Correlation of altered apoptosis and drug resistance with mutations in the p53 gene has shown that inactivation and genetic alterations of the p53 gene is indicative of a worse prognosis in patients with AL. However, a relatively low incidence (10-20%) of genetic alterations in this gene is present in patients with hematological malignancies (Peller et al., 2003). Several enzymes that are involved in apoptosis such as caspase 2 and 3 are expressed at high levels in patients with AL. Survivin and XIAP, members of the "Inhibitors of Apoptosis" proteins family, interfere with activation of caspases, and are expressed in the blasts of AML. However no correlation or prognostic impact of their expression was observed with cytogenetics, remission or overall survival of AML patients. These results suggest caspase-independent mechanisms of cell death in AML (Faderl et al., 2001; Carter et al., 2003). However similar studies in ALL
have failed to confirm the extent of spontaneous apoptosis in vitro, with the response to induction chemotherapy either for B-ALL or T-ALL (Wuchter et al., 2000).

In our study we found that responders and non responders of AML patients showed no significant difference in apoptotic cell count. Although responders of ALL patients showed significantly lower live cell count compared to responders of AML. This suggests different cell biological features of primary AML and ALL cells. In vitro cellular drug resistance is a strong independent adverse risk factor in childhood ALL. Adult ALL samples show significantly more in vitro drug resistance profiles when compared with childhood ALL patients (Styczynski et al., 2000). In our study although responders of patients with ALL showed lower live cell and higher apoptotic cell population compared to non responders, we have found no significant difference in responders of childhood and adult ALL. We also found that patients with ALL who achieved CR were 3.7 times more likely to have mean live cell count less than 70% and 2.7 times more likely to have mean apoptotic cell count >10%.

In an earlier study, no significant differences in drug resistance between adult T-cell and B-cell ALL or adult T-ALL and childhood T-ALL were reported. However, there were significant differences between adult and childhood B-ALL. Lymphoblasts from adults are relatively resistant to drugs commonly used in therapy. This might contribute to the difference in outcome between children and adults with ALL (Styczynski et al., 2000). In the current study such comparison was not done, as the sample size was small. No significant difference was found when the live and apoptotic cell count of B-ALL patients who achieve CR was compared to B-ALL patients who could not achieve CR. Interestingly we found that patients with T-ALL who achieved CR had significantly higher apoptotic cell count compared to patients with T-ALL who could not achieve CR. More recently, microarray data have also suggested several differentially expressed apoptosis related genes between leukemic subgroups defined by lineage, in vitro drug resistant and clinical outcome (Holleman et al., 2006). These data further support our results and show that the expression pattern of apoptotic related genes are strongly suggestive of the possibility of stratifying ALL patients with risk factors.
5.4 IN VITRO CYTOTOXICITY ASSAY:

It has been shown that in vitro evaluation of chemosensitivity of leukemic cell to essential antileukemic drugs has robust predictive power (den Boer et al., 2003). An age-dependent resistance profile has been shown for most antileukemic agents (Pieters et al., 1998). Usually, in vitro drug resistance correlates well with in vivo resistance to antileukemic agents (Kaspers et al., 1998; Schmiegelow et al., 2001). A long-term goal of in vitro chemosensitivity assay is the accurate prediction of patient response to drugs, as it would facilitate the individualization of patient treatment.

ALL: In western developed countries, more than 95% of the children with newly diagnosed ALL achieved complete remission by combination chemotherapy. However, relapse occurs in one third of the patients (Pui et al., 1995). This treatment failure may be explained by unfavorable clinical pharmacokinetics (Evans et al., 1998), regrowth potential of the residual leukemic cells, and by cellular drug resistance (Pieters et al., 1997). Testing of in vitro drug resistance, eg, by using MTT assay as well as flowcytometric assay has provided good correlations with clinical outcome (Bosanquet et al., 1991; Pieters et al., 1991; Hongo et al., 1997; Kaspers et al., 1997). In the present study, in vitro chemosensitivity assay showed that in both B-ALL and T-ALL, Dnr and Vin were the most effective or sensitive drugs while Ara-C, Mtx, and L-asp were the least sensitive drugs. In B-ALL, of the five drugs tested for in vitro chemosensitivity, Vin was found to be correlated with clinical outcome. Leukemic cells from complete responders (CR) were more sensitive in vitro to Vin than non responders (NCR) (70% and 40% respectively, p=0.03). In vitro chemosensitivity of Dnr was 100% in both responders and non responders. In vitro chemosensitivity pattern of other three drugs (Ara-C, Mtx and L-Asp) also showed no significant difference between responders and non responders of B-ALL (p=0.3, 1.0 and 1.0 respectively). However, when the in vitro sensitivity of any of the two drugs in combination was compared between responders and non responders, it was found that patients with CR (responders) were more sensitive to Vin and Dnr (in combination) than NCR (non responders) (p=0.03). Furthermore, none of the prognostic factor in B-ALL was found to be significantly associated with in vitro chemosensitivity of the studied drugs. In T-ALL, such correlation was not done due to small sample size. A trend of better in-vitro sensitivity of Vin was seen in patients...
who had good prognosis factors in both childhood and adult B-ALL. In children, patients showing CD34 positivity \((p=0.2)\) or having low Hb% \(<10\%\) \((p=0.1)\) were more sensitive in-vitro to Vin while in adults, patients showing CD34 negativity \((p=0.5)\), OM (-) \((p=0.05)\) and LAP (-) \((p=0.1)\) (good prognosis factors) were more sensitive in-vitro to Vin. High WBC count has been suggested as an unfavourable factor. In the present study, no significant difference was found in the in vitro sensitivity of Vin between favourable and unfavourable WBC count in both adult \((43\% \text{ vs. } 50\%)\) and children \((60\% \text{ vs. } 64\%)\). A trend of better in-vitro sensitivity for L-asp was also seen in adult patients who were OM (-) \((p=0.1)\) and LAP (-) \((p=0.2)\).

In T-ALL, of the five drugs tested for in vitro chemosensitivity, none of the drug was found to be correlated with clinical outcome. Furthermore, like in B-ALL, in vitro chemosensitivity of Dnr was 100% in both responders and non responders. In vitro chemosensitivity pattern of other four drugs (Ara-C, Vin, Mtx and L-Asp) did not show significant difference between responders and non responders of T-ALL patients \((p=0.2, 0.7, 1.0 \text{ and } 1.0 \text{ respectively})\). In T-ALL, correlation of the prognostic factors with in vitro chemosensitivity was not evaluated as the sample size was small.

In AML: Zwaan et al., (2000) has reported that AML was highly resistant in vitro to most of the drugs. In the present study, in vitro chemosensitivity assay showed that in AML, Dnr and Vin were the most effective or sensitive drugs while Mtx, Ara-C and L-asp were the least sensitive drugs. Kaspers et al., (1994) has reported that AML was more resistant to Vin, which is in contrasts to the result of present study. However, of the five drugs tested for in vitro chemosensitivity, Mtx was found to be correlated with clinical outcome. AML cells from responders (CR) were more sensitive in vitro to Mtx than non responders (NCR) \((67\% \text{ and } 14\% \text{ respectively, } p=<0.01)\). When in vitro sensitivity of any of the two drugs in combination was compared between responders and non responders, it was found that patients with CR (responders) were more sensitive to Mtx and Dnr (in combination) than NCR (non responders) \((p=<0.01)\). In vitro chemosensitivity of Dnr was not found to be correlated with clinical outcome as all the cases from responders and non responders were sensitive to Dnr. In vitro chemosensitivity pattern of other three drugs (Ara-C, Vin and L-Asp) also showed no significant difference between responders and non
responders of AML (p=0.4, 0.7 and 0.5 respectively). Furthermore, in adult AML, patients with CD34- were more sensitive to Ara-C (p < 0.01), Vin (p=0.3) and Mtx (p=0.1), however, in childhood AML no such difference was found. In adult AML, patients with >10% Hb were more sensitive to Ara-C (p= 0.08) and Mtx (p=0.2). These findings confirm the fact that CD34 positivity is poor prognosis factors in adults. In children, a trend of better in-vitro sensitivity of Ara-C was seen in patients who had low WBC count (0.1) or high Plt count (p=0.1) while in adult, a trend of better in-vitro sensitivity of Vin was seen in patients who had low WBC count (0.1) however, in childhood AML no such difference was found. Overall the difference in the pattern of in vitro chemosensitivity assay between childhood and adult AML reveals the difference in the biological behavior of leukemic blasts cells of adult and children.

5.5 EXPRESSION PATTERN OF APOPTOTIC, PROLIFERATION AND CELL CYCLE RELATED PROTEINS AND DRUG RESISTANCE RELATED PROTEINS IN CHEMOSENSITIVE AND CHEMORESISTANT PATIENTS OF AL

Clonal expansion of leukemic cells is caused by proliferation rate (Andreeff et al., 1986) in excess of apoptosis (Wickremasinghe et al., 1999), and several clinical trials have shown that both the proliferative and apoptotic rates of leukemic cells have prognostic significance (Lowenberg et al., 1993; Kornblau et al., 1998). Recent attempts to determine the prognostic variables that differentiate those patients who are likely to achieve complete remission (CR) from those who either achieve partial remission (PR) or fail to respond to current therapeutic regimes have focused on establishing a link between cell survival, cell proliferation and cell death of the neoplastic cells. Failed induction in patients with acute leukemia is commonly due to an inherent or acquired drug resistance because of autonomous proliferation or defects in mechanisms that trigger apoptosis. Chemotherapeutic drugs can kill tumor cells by activating common apoptotic pathways; thus a single mutation that disables apoptosis can induce multidrug resistance. Altered expression of genes encoding key apoptotic proteins can provide cancer cells with both an intrinsic survival and inherent resistance to chemotherapeutic drugs (Johnstone et al., 2002). The overall contribution of apoptotic defects to clinical multidrug resistance remains under
debate. It has also been shown that proliferative activity of AML blasts cell in absence of exogenous growth factors may be associated with poor response to chemotherapy. Thus, in the current study the expression pattern of several apoptosis as well as proliferation and cell cycle related molecules were evaluated in AL patients who responded (chemosensitive) and who did not respond (Chemoresistant) to induction chemotherapy.

Expression of apoptosis related proteins in AML and ALL:

Bcl-2 and Bax are members of the BCL2 family of apoptosis-regulating genes (Reed 1998). Bcl-2 has been reported as an important factor for tumor cell survival and resistance to chemotherapy in hematological malignancies (Hannun 1997). Several studies have shown a correlation between constitutive Bcl-2 expression and response to chemotherapy in AML (Campos et al., 1993; Maung et al., 1994; Lauria et al., 1997; Karakas et al., 1998; Wuchter et al., 1999). Norgaard et al., (1996) has also reported that high blast survival is associated with poor response to chemotherapy. In the current study, AML patients who did not respond (NCR) to chemotherapy showed higher mean% of Bcl-2+ cells compared to responders (CR). Increased expression of BCL-2 at mRNA level was also observed in non responders compared to responders (6.3 fold and 1.7 fold respectively). These results are similar to Bincoletto et al., (1999) and Karakas et al., (1998) who have reported higher Bcl-2 expression in the chemotherapy resistant group. However, in contrasts to this, Naumovski et al., (1998) has shown no association between Bcl-2 expression and clinical outcome. Furthermore, in the current study, Bcl-2 positive cases were more resistant in vitro to Ara-C, Vin, Mtx and L-asp drugs compared to Bcl-2 negative cases. In addition to this Bcl-2 was found to be associated with high WBC count and CD34 positivity (adverse prognostic features in AML). Konopleva et al., (2002), van Stijn et al., (2003) and Venditti et al., (2004) has also shown the overexpression of the Bcl-2 in CD34+ AML cells and suggested their involvement in the chemoresistance. These results suggest that CD34+ fraction is more resistant to apoptosis than the corresponding CD34- fraction due to higher expression of Bcl-2. In ALL, Bcl-2 positivity was not found to be correlated with clinical outcome, in vitro chemosensitivity assay and prognostic factors. These findings are similar to results of Salomons et al., (1999) and Wuchter et al., (2000) who have also reported
that expression levels of the Bcl-2 family do not correlate with the response to induction chemotherapy, in vitro drug resistance and relapse rate in childhood ALL. The loss of Bax (Bcl-2-associated X protein) expression is a frequent event in patients with solid tumors and a negative prognostic factor for therapeutic response Sturm et al., (1999). Caspases are one of the key effector molecules in apoptosis. Inappropriate expression of caspases or malfunctions in their regulation through other pathways may also be an important step in the pathogenesis of acute leukemia. In the present study, AML patients who not respond to chemotherapy showed lower mean% of Bax+ cells and caspase3+ cells compared to responders. Ong et al., (2000) and Del Poeta et al., (2003) have also reported that low expression of Bax is a poor prognostic indicator in AML. However, Bax was not found to be associated with in vitro chemosensitivity assay as well as any prognostic features. Caspase-3 positive cases were found to be more sensitive to Ara-C and L-asp and showed association with good prognostic features i.e. Auer rods positivity, low WBC count and CD34 negativity. Earlier studies have also reported that caspase 3 (Fernandes-Alnemri et al., 1994; Nicholson et al., 1995; Tewari et al., 1995) involved in the apoptosis of leukemic cell induced by various cytotoxic agents such as Ara-C (Datta et al., 1996; Ibrado et al., 1996). Furthermore, caspase3 has been reported as a possible prognostic factor in AML by Oliver et al., (2002). As in AML patients, non responders of ALL had lower mean% of Bax+ and caspase3+ cells compared to responders. Furthermore, in the present study, Bax+ cases were relatively more in vitro sensitive to the tested drugs than Bax- cases. No association of Bax was found with the prognostic factors. For Bax, conflicting findings has been reported in the literature, on one hand decreased Bax expression has been shown to be associated with an increased risk of relapse of ALL (Prokop et al., 2000) while on the other hand increased Bax has been shown to be associated with an increased risk of relapse in ALL (Hogarth et al., 1999). Caspase-3 has been determined to be a significant predictor of complete remission in ALL (Faderl et al., 1999). Faderl et al., (2001) has shown that over expression of inactive form of caspases is frequently observed in ALL. In the present study, caspase-3 positivity was found to be associated with low WBC count. This finding suggests that lack of expression of caspase-3 due to abnormality in apoptotic pathway may be a reason for high WBC count in AL.
Expression of proliferation and cell cycle related proteins in AML and ALL:

Ki67 is widely recognized marker of proliferation as it is present only in proliferating cells (Gerde et al., 1984) while cyclin D1 proto-oncogene is an important regulator of G1 to S-phase transition in numerous cell types from diverse tissues. Moreover, cyclin D1 has also been shown to act a cofactor for several transcription factors. Jaroslav et al., (2005) has shown no association between cyclin D1 expression and clinical outcome in AML. However, Li et al., (1999) has shown that overexpression of cyclin D1 in acute leukemia patients is correlated with the disease progression, especially with relapse. The present study also confirms that mean% of cyclin D1 was higher in non responders compared to responders. Increased expression of cyclin D1 at mRNA level was also observed in non responders compared to responders (2.1 fold vs. 0.04 fold). Furthermore, in vitro chemosensitivity assay also showed that cyclin D1 positivity was associated with resistance to Ara-C, Vin, Mtx drugs. However, cyclin D1 was not found to be associated with any prognostic factors. Lowenberg et al., (1993) has shown that proliferative activity of AML blasts cell in absence of exogenous growth factors might be associated with poor response to chemotherapy. However, in the present study, there was no difference in the mean Ki-67 positive cells between non responders and responders and furthermore Ki-67 was not found to be associated with the in-vitro chemosensitivity assay and with any prognostic clinical features. In ALL, mean% of cyclin D1 was higher in non responders compared to responders. Volm et al., (1997) has reported a lower probability of remission in cyclin D1 positive cases compared to cyclin D1 negative or weakly positive cases. Furthermore, in vitro chemosensitivity assay showed a trend between cyclin D1 positivity and resistance to Vin and Mtx drugs. No association was seen between cyclin D1 positivity and prognosis factors however, Aref et al., (2006) showed that cyclin D1 positivity was associated with lymphadenopathy (LAP). In the present study, there was no difference in the mean ki-67 positive cells between NCR patients and CR patients. However, interestingly it was found that ki-67 positivity was associated with the in-vitro chemosensitivity to Vin. No association was seen between Ki-67 positivity and prognostic factors. P21 protein, regulated transcriptionally by the P53, acts both as inhibitors of CDK activity and thus is
important for cell cycle control (LaBaer et al., 1997; Reynisdottir et al., 1997). In the current study, in both ALL and AML, no significant difference was seen for p21+ cells in non responders and responders. Furthermore, no significant association was seen between p21 positivity and in vitro chemosensitivity as well as prognosis factors.

The P53 tumor-suppressor gene integrates numerous signals that control normal cell proliferation and apoptosis (Vogelstein et al., 2000). Mutation of the P53 tumor-suppressor gene is one of the most common molecular alterations in a variety of tumors (Hollstein et al., 1999), but it occurs infrequently in ALL (5%) (Kawamura et al., 1999) and AML (9%) (Stirewalt et al., 2001). An additional proposed mechanism for inactivation of P53 protein is the amplification of the Mdm2 and its overexpression (Finlay et al., 1993). The activity of P53 and the expression of MDM2 are coregulated in auto regulatory feedback loop (Shieh et al., 1997). Overexpression of MDM2 mRNA has been reported in 42% of ALL and 53% of AML, but in the absence of MDM2 gene amplification (Bueso-Ramos et al., 1993). It has been reported that MDM2 has the potential to interact with pRB and E2F pathways to possibly promote transformation (Xiao et al., 1995). In the present study, p53 expression was low in both ALL and AML. However, in non responders of AML, p53 positive cells was more common compared to responders while no such difference was seen between non responders and responders of ALL. Interestingly, in both AML and ALL, non responders had higher Mdm2 positive cells compared to responders suggesting that overexpression of Mdm2 might be responsible for the elimination of p53 tumor suppressor function which leads to resistance to chemotherapy. Furthermore it was found that MDM2 positive cases were more resistant to Mtx than Mdm2 negative cases in AML. Zhou et al., (1995) has also found overexpression of the Mdm2 in ALL cells expressing the wild-type p53 gene. These findings suggest an important role for Mdm2 in the pathogenesis of AL. Seliger et al., (1996) has also shown that structural alterations of the p53 gene do not play an important role in the initiation and progression of AML. However, abrogation of p53 tumor suppressor function due to MDM2 overexpression may be an alternative molecular mechanism by which a subset of AML may escape from p53-regulated growth control.
Expression of drug resistance related proteins in patients with AML and ALL:

Although the development of new therapeutic agents and treatment regimens has improved the cure rate of acute leukemia, >50% of adult patients relapse and do not survive (Arlin et al., 1992; Buchner et al., 1997). One of the major obstacles to successful treatment is the drug resistance of leukemic cells to chemotherapeutic agents. The multidrug resistance has been shown to be partly mediated by the enhanced expression of the multidrug resistance 1 (MDR1) gene (van den et al., 2000), which encodes P-glycoprotein (Pg-p) (Malayeri et al., 1996; Zubercova et al., 1998). In the present study, it was found that mean% of P-gp was higher in non responders compared to responders in both ALL and AML. These findings are similar to other studies, which also report that high Mdr1 proteins activity in AML is associated with poor response to chemotherapy and reduced patient’s survival (Pirker et al., 1991; Leith et al., 1997; Leith et al., 1999; Laupeze et al., 2002). In ALL, however conflicting results have been reported. Some studies have found a prognostic impact Mdr1 expression in childhood ALL (Sauerbrey et al., 1994; Dhooge et al., 1999), but others failed to show such an association (den Boer et al., 1998; Kanerva et al., 2001). Furthermore, in vitro chemosensitivity assay showed a trend between Mdr1 positivity and more resistance to Ara-C, Vin and Mtx drugs in both ALL and AML. Important agents in the treatment of leukemia, including anthracycline, Vinca alkaloids, and podophyllins, have been identified as substrates for P-gp (Sonneweld et al., 2000; van den et al., 2000). Mdr1 expression was also found to be associated with higher Hb% and CD34 negativity in AML. Similar to this, (Venditti et al., 2004) has also showed that Mdr1 is overexpressed in CD34- cells in AML. Several other studies have also shown an association between Mdr1 expression or Mdr1 function and a CD34 positive immature phenotype in acute myeloid leukemia (AML) (Campos et al., 1992; Sandani et al., 1996; Legrand et al., 1998; Wuchter et al., 1999). Although other studies did not confirm these data (Del Poeta et al., 1996; Zochbauer et al., 1997).

Dihydrofolate reductase (DHFR) is an enzyme involved in the metabolism of nucleic acids; it is also an important target for folate antagonists such as methotrexate (MTX). Dihydrofolate reductase (DHFR) gene amplification has been reported as a common mechanism of resistance to methotrexate (MTX) in tumor cell lines, with
the exception of a few case reports. Goker et al., (1995) has shown that amplification of DHFR gene is a mechanism of acquired resistance to Mtx in AL. In the present study, it was found that mean% of DHFR was marginally higher in non responders compared to responders in AML while such difference was not seen in ALL. Furthermore, in vitro chemosensitivity assay showed that all patients showing DHFR positivity were resistant to Mtx in both ALL and AML. DHFR was not found to be associated with with any prognostic clinical features in ALL or AML. However, Matherly et al., (1997) has suggested that DHFR overexpression is associated with the poor prognosis of patients with T-ALL treated with standard dose antimetabolite therapy.

GST-pi belongs to a major group of detoxification enzymes that is widely distributed in the human body. GST over-expression has been reported in anthracycline selected multidrug resistant cell lines (Tew 1994). In the present study, it was found that mean% of GST-pi+ cells was marginally higher in non responders compared to responders of AML while such difference was not seen in ALL. However, Sauerbrey et al., (1994) has shown that overexpression of GST-pi was associated with a higher relapse rate and a lower probability of remaining in first remission in ALL. Furthermore, GST-pi was not found to be associated with in vitro chemosensitivity assay and any prognostic features in ALL or AML. No difference was seen in the expression level of GST-mRNA between non responders and responders of ALL and AML. However, Tiedfelt et al., (1992) has suggested that GST activity and GST mRNA are useful for predicting the chemosensitivities and the prognosis of the disease.