

and immuno-fluorescence microscopic slides (Biomérieux, USA). Fluorescence microscope was used with 40 X-magnification lenses at 490 nm to examine the slides immediately or 1-3 days later as a maximal duration. Detection of positive cells by observing a dot-like apple green fluorescent colored light (FITC) and red fluorescent colored light (PE) on the surface of nTregs. Whereas, CD4+ T cells were detected by observing only a dot-like apple green fluorescent colored light (FITC) on the surface of positive cells.

### Statistical Analysis

The percentage of both nTregs and CD4+ T cells was measured by counting the number of positive cells/field in 5 to 10 microscopic fields to the total lymphocyte count as follows:

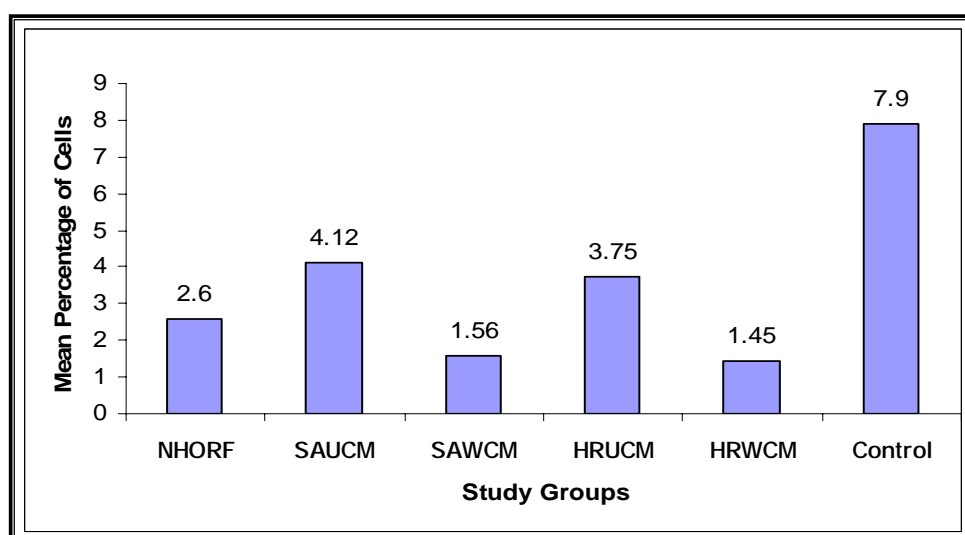
The percentage of positive cells = *the number of positive cells / the number of total cells X 100*.

The correlation coefficient (*r*) was calculated as a quantitative descriptive to the association between the mean percentage of CD4+ T cells and CD4+CD25+ nTreg cells among different study groups.

All statistical analysis was performed with the SPSS 10.01 statistical package for social sciences and also Excel 2003. A *p* value of less than 0.05 ( $p < 0.05$ ) was considered significant.

### Results

The percent of naturally occurring CD4+CD25+ cells was evaluated by immunofluorescence staining technique and the results shown in (Table 1 & Figure 1) displayed the difference in the mean percentage among all groups under study. High risk groups (HR<sup>UCM</sup> and HR<sup>WCM</sup>) displayed lower CD4+CD25+ regulatory T cells expression than single attack groups. In HR<sup>WCM</sup> patients, lower percent of nTregs was recorded (1.45%) when compared with SA<sup>WCM</sup> group (1.56%) and this difference was also found between groups of continuous medication (HR<sup>UCM</sup> and SA<sup>UCM</sup>) (3.75% and 4.12% respectively). These results refer to the difference between groups of continuous medical care which displayed higher numbers of nTregs than patients of intermittent medical therapy.



**Figure1: Mean percentage of peripheral blood nTreg cells among different study groups**

Negative history (NH) group had high mean percentage (2.6%) than both HR<sup>WCM</sup> and

SA<sup>WCM</sup> groups. In general, CD4+ CD25+ nTregs were found in lower numbers in the