MATERIALS & METHODS

STUDY CONCEPTS
To develop a non-invasive method based on quantitative ultrasound image analysis to characterize tissue composition in terms of intensity and structure in and around shoulder joint by using histogram analysis. Various shoulder pathology analyses were studied by high resolution ultrasound, but none of them will characterize the tissue. Hence we used histogram to analyze tissue composition in terms of intensity.

STUDY DESIGN
Patient selection
One hundred and forty nine patients with shoulder pain who were referred for high resolution ultrasound by the Department of Orthopaedics, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, Chennai-116 were recruited for this study. The study was performed by sonoradiologist of Department of Radiology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University Hospital from October 2003 to November 2007. Patients with obvious shoulder or clavicular fracture or dislocation or previous surgery were excluded. Patients with clinically suspected shoulder pathology were recruited and included in the study when typical clinical signs were
demonstrated in ninety two men and fifty seven women in the age group of 11 -68 years.(mean age 35.74 years ± 11.18).The duration of shoulder pain symptoms was less than 24 months.

All patients were examined clinically by orthopedic surgeons. In addition, the patients answered questions to assess physical function – that is, the ability to move upper limb.

These questions focused on the ability of the patients to perform such functions as flexion, extension, internal rotation and external rotation of the shoulder and on the basis of the answers to these questions, the overall scores were determined.

Before the study commenced, the design protocol was approved by the hospital health ethics and research committee. Informed consent was obtained for all patients after the nature of the ultrasound examinations had been fully explained. Shoulder ultrasound examinations were done on both sides on the same day. All shoulder examinations were performed in accordance with the American College of Radiology Guidelines of the shoulder.
SONOGRAPHY STUDIES

Ultrasonography was performed by the experienced sonoradiologist who was unaware of the patient’s history or findings of the physical examination. The ultrasound images used in this study were obtained by ALOKA PRO SSD 5500 Scanner Ultrasound Machine. High resolution linear 5 – 10 Mhz linear probe was used (PLATE 5). 512 x 512 image frames with gray level resolution of 8 bits/pixel. Inbuilt software tools on this system to allow the sonoradiologist to define the region of interest (ROI) for histogram in the image for further analysis.
High resolution ultrasound equipment with linear transducer
During ultrasonic examination, the patient was seated in revolving chair and the sonoradiologist stood behind the patient during the scan. Ultrasonography of the shoulder begins with a transverse and longitudinal image of the biceps tendon within the bicipital groove. Next, longitudinal and transverse scans of the Subscapularis tendon are made with the patient’s arm externally rotated. Images of the supraspinatus tendon are made with the arm in internal rotation to expose as much of the supraspinatus as possible from beneath the acromion. This position is best achieved by placing the patient’s arm behind his or her back. Alternatively, keeping the hand in the waist depressing it as much as possible to adequately study the deeper fibers of supraspinatus muscle. The supraspinatus tendon may be scanned perpendicular and parallel to its fibers. The thickness and echogenicity of the tendon, the segmental or complete loss of rotator cuff substance, the presence and amount of joint and bursal fluid, the loss of contour of the tendon changes are observed. Histogram values at the above areas also obtained. In addition, it is important to use the transducer to compress the deltoid muscle against the cuff in an attempt to separate the torn tendon ends at the site of a non retracted tear. Dynamic action shoulder also performed to delineate the lesions well. The standardized Ultrasonographic criteria for rotator cuff evaluation were used in each patient.
DATA ACQUISITION

The procedure of choosing the ROI and parameter calculation was performed under different imaging conditions such as scanning frequency, machine settings (TGC, gain, contrast and zooming), in addition to varying the size and location of the ROI. The following scanning conditions must be standardized for all scans to ensure the fidelity of the tissue characterization procedures.

1) Ultrasound machine settings which change the overall image gain and produce uncontrollable effects. It is important that the same settings are used for all tissues, to avoid deviations in the image statistics. Also, the transducer type should be the same for all cases to avoid any bias between different interpolation schemes used for different scan protocols. Moreover, the frequency of ultrasound waves used must be the same since the attenuation of ultrasonic waves depends mainly on this frequency.

2) ROI size and shape. To obtain reliable statistics, the number of pixels in the ROI must be at least 800 pixels to provide the suitable sample size condition for reliable statistics. Also, a square shape of the region should be maintained during all measurements.
3) ROI location. To avoid the distorting effects in ultrasonic wave patterns, such as side lobes and grating lobes, the particular region should be selected each time along the centre line of the image. Also, the depth of the region of interest should be chosen such that the distorting effects of the reverberations in the shallow parts and the attenuation in the deep parts are avoided.

Transmitting ultrasound beams in a scan plane. Detecting ultrasound echoes reflected at a multiplicity of sample volumes in the said scan plane. Acquiring raw acoustic sample data derived from ultrasound echoes reflected from the said scan plane. Storing an image frame data set of raw acoustic sample data for said scan plane. Organizing the said raw acoustic sample data of the said image frame data set into a histogram data set. Determining a first end point of a map input range as a function of the said histogram data set. Determining a second end point of the said map input range as a function of the said histogram data set. Constructing a gray-scale map comprising output values corresponding to respective input values of the said map input range. Applying the said gray-scale map to an image frame data set to form a gray-scale mapped image frame data set and displaying the said gray-scale mapped image frame data set.
Image processing often times involves mathematical operations performed on histograms characterizing the images. In image processing context, a histogram typically refers to a graph showing the number of pixels in an image at each different intensity value found in that image. For example, for an 8-bit gray-scale image, there are $2^8 = 256$ different possible intensity values, and the histogram graphically displays 256 numbers showing the distribution of pixels amongst those intensity values.

The histogram constructor comprises an average calculator, for calculating, for each picture-element of the plurality of picture-elements, a set-averaged intensity value, thereby to provide an average intensity matrix representing the moving image and to construct the intensity histogram using the average intensity matrix.

The major artefact in ultrasound images is clutter, which includes irrelevant information that appears in the imaging plane, obstructing the data of interest. There are several causes for the appearance of clutter in an ultrasonic image. A first cause is effective imaging of off-axis objects, primarily due to highly reflective objects in the transducer's side lobes.

A second cause is known as multi-path or reverberations. Due to the geometry of the scanned tissue with respect to the transducer, and the local reflective characteristics of the tissue, a substantial amount of the transmitted
energy is bounced back and forth in the tissue before reaching the transducer. As a result, the signal measured at a specific range-gate includes contributions from incorrect ranges, in addition to the relevant range.

A two Dimensional ultrasound image usually consists of a rectangular array of tiny squares called ‘Picture elements’ or pixels for short. Associated with each pixel is a number, representing the average brightness of that part of the original picture covered by the pixel. Usually, the brightness will be discredited to 8-bit resolution, i.e., there are $256 = 28$ shades of gray, with 0 representing black and 255 representing white. The pixel brightness may represent any variation which has been measured on a 2D grid. Typically it is a measure of the intensity of reflected light. The histogram corresponds to the estimated probability density function of the noisy data.
STATISTICAL ANALYSIS METHODS

SPSS 11.0 statistical software (Chicago IL) was used for all statistical analyses, K-related samples and Kendall’s (coefficient of concordance) non-parametric tests were selected for comparison of the physical examinations and Ultrasonographic findings. We accepted high resolution ultrasound as the gold standard. The true and false, positive and negative cases were recorded. Calculations were performed to determine sensitivity and specificity of selected clinical tests and Ultrasonographic findings.

SET OF PARAMETERS USED FOR STATISTICAL ANALYSIS:

1) False-negative rate is defined as the probability that the classification result indicates a normal while the true diagnosis is indeed the disease (i.e., positive). This case should be completely avoided since it represents a danger to the patient.

2) False-positive rate is defined as the probability that the classification result indicates a disease while the true diagnosis is indeed a normal (i.e., negative). This case can be tolerated, but should be as infrequent as possible.
3) Sensitivity is defined as the conditional probability of detecting a disease while there is in fact a disease. By definition, Sensitivity = 1 - false-negative rate.

4) Specificity is defined as the conditional probability of detecting a normal rotator cuff while the rotator cuff is indeed normal. By definition, Specificity = 1 - false-positive rate.