CHAPTER 3

WISTAR RAT – BREAST CANCER MODEL

3.1 BACKGROUND

Animal surface temperature profile captured using infrared camera is helpful in the assessment of physiological responses associated with the regulation of body temperature. Diagnosing breast cancer in early stage itself has a greater effect upon the prognosis. In Digital infrared imaging, diagnosis is based upon the principle of analyzing temperature variation associated with metabolic activity and vascular circulation changes in the affected area rather than anatomical changes [69]. IR imaging is being extensively used for diagnosis, to study the progress of treatment of many disorders, mainly in the wide area of rheumatology [70], dermatology, orthopedics and circulatory abnormalities [14]. Thermography was widely used in diagnosis, prognosis of several diseases and to study progress of treatment with animal model. Capsaicin can be used as a potential therapeutic adjuvant which significantly enhances the heating effects of biological tissues during tumor hyperthermia. Increase in temperature induced by intra peritoneal injection of capsaicin was more obvious than the other two methods of locally smeared with and subcutaneous injection of capsaicin [71]. Dynamic thermal imaging has been used intraoperatively during neurosurgical interventions, including real-time assessment of cerebral vessel patency and cerebral perfusion, monitoring of flap perfusion and determination of tumor margins during resectional surgery [72, 73 & 74]. The interpretation of cancer in human was proven on scoring 5 IR signs by the analysis of ROC (Receiver Operating Characteristic) curve and AUC (Area Under the ROC Curve) by the univariate logistic regression model for each IR sign and an age-adjusted multivariate logistic regression model by Wang et al (2010)
[13]. Motta et al (2010) designed an automatic approach to eliminate human factors and guarantee repeatability and robustness of the results and by preventing errors caused by fatigue [76]. Thermographic imaging could detect temperature changes as small as 0.1°C decrease on the skin surface at an early stage of tumor and it was proven in Xenografts (MDAMB-231, MCF7) induced nude mice model [15]. In contrast, the temperature and thermal conductivity in the cancerous breast were higher than the normal breast in human due to angiogenesis and higher metabolic rate of cancerous cells [77–79].

13762 MAT mammary adenocarcinoma animal model was used for examining the effects of subcutaneous tumor growth on skin temperature and whether angiogenesis-induced changes in vascular density play a role in any surface temperature changes [80]. DMBA induced animal model closely mimics human breast cancer as it actively metabolites with a capacity for damaging the DNA molecule, the main event in carcinogenesis initiation [81-83]. Selenium in serum and neoplastic tissue in breast cancer was correlated with serum CEA level to analyze tumor growth [84]. Serum CEA (Carcino Embryonic Antigen) levels were measured to check the clinical improvement and to study the antitumour activity of histone H1 [85]. The aim of this study was to investigate the potential of thermography in the evaluation of breast cancer in wistar rat model.

The objectives of the study were as follows: i).To induce breast cancer in the lower-right flank region of wistar rats (n=6) using DMBA (7,12-dimethylbenz(a)anthracene); ii).To capture thermal images of rats for 60 days from the day of induction until the tumor becomes into a palpable size; iii).To investigate asymmetrical distribution of skin surface temperature (°C) as well as pseudo color distribution between breast cancer induced lower right flank region and non tumor site; iv).Asymmetrical RGB color histogram analysis; v).To compare the thermal study results, and biomarker carcino embryonic antigen (CEA) results and biopsy as standard.
Figure 3.1 Study design: wistar rat- Breast cancer model

Female wistar rats (n=8)
- Group – I (n=6): Chemical carcinogen DMBA induced rats
- Group –II (n=2): Normal control rats

Group–I (n=6)
Induced tumor at right lower flank region using DMBA

Group–II (n=2)
Normal

Whole-body thermogram using FLIRT400 thermal camera for 60 days

1. Thermogram image analysis
   a) Measurement of average SST at specific ROIs using FLIR 1.2 Quick report
   b) Asymmetrical SST analysis
   c) RGB color histogram analysis of ROIs

Biochemical test
(CEA)
(at the end of the study)

Biopsy
(at the end of the study)
3.2 MATERIALS AND METHODS

3.2.1 Study design

The study design for wistar rat breast cancer model is shown in Figure 3.1.

3.2.2 Laboratory animal study design

Female wistar rats (n=8) weighing 300-350g and aged between 12 to 13 weeks were obtained from Kings Institute of Preventive Medicine Guindy, Chennai, India. The animal experimental protocols used for the study were approved by SRM animal experimentation ethics committee, SRM University, Kattankulathur, Chennai, India. The animals were caged individually and kept at constant environmental and nutritional conditions throughout the experimental period with room temperature 23±2°C, humidity(50-55%) with 12h light/12h dark cycle and were fed a standard pellet diet and with water adlibitum.

The rats were divided into the following groups:

**Group I** : Chemical carcinogen dimethyl benza anthracene (DMBA) induced rats (n=6)

**Group II** : Normal control rats (n=2).

3.2.3 Breast cancer induction by chemical carcinogen

20 mg of 7, 12-dimethyl benz (a)nthracene (DMBA) purchased from Sigma chemicals distributors in Chennai, India was mixed with 0.5 ml of sunflower oil and 0.5 ml of saline to induce breast cancer in each rat. A single-dose injection of DMBA was given subcutaneously into the right flanks only in the test Group-I. 0.5ml of sunflower oil and 0.5ml of saline alone injected into the left flanks control side in all six rats, and Group-II animals (control) were injected with 0.5ml of sunflower oil and 0.5ml of saline in both right and left flank region.
3.2.4 Thermogram acquisition

AP view of whole body thermal images of the rats were taken under standard conditions at constant distance of (12cm). Every day after one hour acclimatization of wistar rats in the temperature controlled recording room, the images were captured with constant temperature of 23.3°C, at 12.00 noon (Indian time). On zeroth day at 12 noon, AP view of whole-body thermal images of all the rats of Group - I (breast cancer induced rats before chemical carcinogen induction) and Group - II (normal control rats) were taken under standard conditions. On the first day, at 11.30 AM 20mg of DMBA, 0.5ml of sunflower oil and 0.5ml of saline was injected at the right side of flank region of all the six rats in Group-I, whereas 0.5ml of sunflower oil and 0.5ml of saline alone were injected in both right and left flank regions of 2 rats in Group - II. All the animals were allowed to acclimatize for 30 minutes. After half an hour (at 12.00 noon), thermal images was acquired as like zeroth day. Following this, every day over a period of nine weeks, thermal images were captured at 12.00 noon using the standard protocol explained in Chapter 2, Section 2.4.3.2.

3.2.5 Thermogram SST (°C) analysis

For asymmetrical SST (°C) distribution analysis, the whole thermal image of rat was divided into six square ROIs as follows: i).the shoulder region: a).right shoulder and b).left shoulder; ii).abdomen region: a).right abdomen and b).left abdomen; and iii).lower flanks region: a).right- and b).left- flank. The methodology was explained in detail in Chapter 2, Section 2.4.3.3. An average SST (°C) distribution of the selected ROIs was measured using FLIR software and RGB color histogram of the ROIs was plotted using MATLAB as discussed in Chapter 2, Section 2.5.5.

3.2.6 CEA test

2 ml of blood was collected from each rat after the tumor had developed into a palpable size and serum was separated. CEA levels (ngm/ml) in the serum of
experimental animals were measured as per the procedure described in Chapter 2, Section 2.1.2.1.

3.2.7 Histopathology study

All the tumor induced rats were sacrificed after 90 days, i.e., the tumor had developed into a palpable size. The induced tumor was removed from rat, and the mammary cancer tissues were cut. The same was prepared as slides as explained in detail in Chapter 2, Section 2.2.2.1 and histopathology of the tumor tissue was studied.

3.2.8 Reproducibility of average SST (°C) measurement

The whole body thermal images of the wistar rats taken on 25\textsuperscript{th} day of the study duration were randomly chosen for analysis of reproducibility. The images of both a control as well as experimental rats were chosen blindly. In the image six ROIs were selected as explained in Chapter 2 of Section 2.4.3.3. The measurement of average SST (°C) in the selected ROIs was done using FLIR 1.2 quick report software with the following VI cases of study. In study I, the following variables were kept constant: i).area of ROI; ii).shape of ROI; iii).position of ROI; and iv).day of measurements made, whereas the time of measurements made was varied as mentioned below: i).9.00 AM; ii).12.00 noon; and iii).5.00 PM. In study II, the following variables were kept constant: i).shape of ROI; ii).position of ROI; iii).day of measurements made; and iv).time of measurements made whereas the areas of ROIs were varied as mentioned below: i) 1× 1 cm; ii).1.5 × 1.5 cm; and iii).2 × 2 cm. In study III, the following variables were kept constant: i).fixed area of ROI (1× 1 cm); ii).shape of ROI; iii).day of measurements made; and iv).time of measurements made whereas the position of ROI was slightly varied as mentioned below: i).upward; ii).downward; iii).right side; and iv).left side to the correct position of ROI. In study IV, the following variables were kept constant: i).fixed area of ROI (1.5× 1.5 cm); ii).shape of ROI; iii).day of measurements made; and iv).time of measurements made whereas the position of ROI was slightly varied as
mentioned below: i). upward; ii). downward; iii). right side; and iv). left side to the correct position of ROI. In study V, the following variables were kept constant: i). fixed area of ROI (2×2 cm); ii). shape of ROI; iii). day of measurements made; and iv). time of measurements made whereas the position of ROI was slightly varied as mentioned below: i). upward; ii). downward; iii). right side; and iv). left side to the correct position of ROI. In study VI, the following variables were kept constant: i). area of ROI; ii). shape of ROI; iii). position of ROI; and iv). time of measurements made whereas day of measurements made was varied as mentioned below: i). day 1; ii). day 2; iii). day 3; iv). day 4; v). day 5; vi). day 6; and vii). day 7. The inter observer error in the average SST (°C) measurement was calculated by finding the value of technical error of measurement (TEM) between two average SST (°C) measurements. TEM is the standard deviation between repeated measurements taken independently by one observer (intra-observer). TEM was calculated using the Equation 3.1.

\[
\frac{\sqrt{\sum d_i^2}}{2n} \tag{3.1}
\]

where: ‘d’ is the difference between measurements and ‘n’ is the number of times measured.

Coefficient of reliability, R which ranges from 0 to 1, estimates the proportion of the inter-subject variance (total measurement variance) that is not due to measurement error and is given by the Equation 3.2.

\[
R = 1 - \frac{TEM^2}{SD^2} \tag{3.2}
\]

where TEM is technical error of measurement and SD is standard deviation of the sample. It reveals the proportion of between-measurement variance in a measured region which is free from measurement error.

3.2.9 Statistical analysis

Mean and standard deviation of SST (°C) measured in six ROIs of all the rats in the group-I was calculated for every week. Student’s ‘t’ test was performed to
analyze the difference in symmetrical SST (°C) distribution between right- and left-regions of selected ROIs. Serum CEA levels were also reported as mean ± SD for (n=6) group-I and group-II (n=2). All these statistical calculations were carried out using SPSS 10.0 software.

3.3 RESULTS

3.3.1 SST (°C) distribution in tumor induced lower flank region

Mean and standard deviation of SST (°C) for different ROIs were calculated every week and tabulated. Table 3.1 shows the SST (°C) distribution comparisons between tumor induced lower right flank region and control lower left flank region. In the base line image, i.e., before the chemical carcinogen induction, calculated mean and SD values of SST (°C) at right flank and left flank were 38.98±0.28 and 39±0.13 respectively. Percentage asymmetrical SST (°C) difference between right- and left- flanks was 0.05% and there was no significant difference (i.e. p=0.854) between right and left flank regions. It showed symmetrical SST (°C) distribution in the flank region before chemical carcinogen induction. Immediately after chemical carcinogen induction mean and SD values of SST (°C) at right flank and left flank were 36.62±0.2 and 36.8±0.24 respectively and SST (°C) difference of 0.49% were observed. However, there was no significant difference (i.e. p=0.105) between right- and left- flank region. After the first week of induction significant SST (°C) difference (<0.001) was observed every week until ninth week, which indicates the asymmetrical SST (°C) distribution within the tumor induced lower flank region. SST (°C) difference about (0.2 to 0.6 °C) was found within the tumor induced region between lower right- and left- flank regions. % asymmetrical SST variation observed was 0.5% to 2%. Greater difference in SST of 1.73% was observed in the ninth week.

3.3.2 SST (°C) distribution in non tumor abdomen region

Similarly SST (°C) distributions on the right- and left- abdomen region were also compared. From Table 3.1, it was observed that there was no significant
difference in SST (°C) distribution between the right- and left- abdomen region, before chemical carcinogen induction (p=0.43), immediately after carcinogen induction (p=0.76) and also after the tumor induction in all the weeks throughout the study period. Percentage asymmetrical SST difference was <0.51 % in all the weeks. The SST difference was very less i.e. < 0.19 °C.

3.3.3 SST (°C) distribution in non tumor shoulder region

SST (°C) distributions on the right and left shoulder region were also compared (Table 3.1). There was no significant difference in SST (°C) distribution between the right shoulder and left shoulder region, before chemical carcinogen induction, i.e. in the baseline thermal image (p=0.56), immediately after carcinogen induction (p=0.461) and also after the tumor induction in all the weeks throughout the study period. Percentage asymmetrical SST difference was <0.44 % in all the weeks. The SST (°C) difference was very less i.e. <0.16 °C.

Average SST (°C) of all the tumor induced groups calculated weekly was plotted separately in all the three regions (flank, abdomen and shoulder region) to visualize right and left-side SST (°C) variations. From Figures 3.2.b and 3.2.c it was clear that in abdomen and shoulder region, right and left-side mean SST (°C) curves were overlapping whereas in tumor induced flank region difference in SST (°C) distribution was found (Figure 3.2. a), between the right- and left- sides in all the weeks.

Percentage asymmetrical SST distribution, over the period of study until the ninth week of the entire region was plotted. From Figure 3.3, the SST variation between tumor induced lower right and left flank region was about 0.5 to 2%, whereas the SST variation was very less in the other region (abdomen and shoulder region) about less than 0.5% throughout the period of study, which clearly indicates that asymmetrical SST (°C) distribution got in tumor induced lower flank region where as other abdomen and shoulder regions were symmetrical.
Table 3.1 Comparison of mean SST (°C) region wise (flank region, abdomen region, shoulder region)

<table>
<thead>
<tr>
<th>Week</th>
<th>Tumor induced site – Lower flank region</th>
<th>Non tumor site - Abdomen</th>
<th>Non tumor site – Shoulder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower right flank region SST (°C)-Mean ± SD</td>
<td>Lower left flank region SST (°C)-Mean ± SD</td>
<td>Difference in SST between right flank and left flank region (°C)</td>
</tr>
<tr>
<td></td>
<td>Lower right flank region SST (°C)-Mean ± SD</td>
<td>Lower left flank region SST (°C)-Mean ± SD</td>
<td>Difference in SST between right flank and left flank region (°C)</td>
</tr>
<tr>
<td></td>
<td>Lower right flank region SST (°C)-Mean ± SD</td>
<td>Lower left flank region SST (°C)-Mean ± SD</td>
<td>Difference in SST between right flank and left flank region (°C)</td>
</tr>
<tr>
<td></td>
<td>Lower right flank region SST (°C)-Mean ± SD</td>
<td>Lower left flank region SST (°C)-Mean ± SD</td>
<td>Difference in SST between right flank and left flank region (°C)</td>
</tr>
<tr>
<td></td>
<td>Lower right flank region SST (°C)-Mean ± SD</td>
<td>Lower left flank region SST (°C)-Mean ± SD</td>
<td>Difference in SST between right flank and left flank region (°C)</td>
</tr>
<tr>
<td></td>
<td>Lower right flank region SST (°C)-Mean ± SD</td>
<td>Lower left flank region SST (°C)-Mean ± SD</td>
<td>Difference in SST between right flank and left flank region (°C)</td>
</tr>
<tr>
<td></td>
<td>Lower right flank region SST (°C)-Mean ± SD</td>
<td>Lower left flank region SST (°C)-Mean ± SD</td>
<td>Difference in SST between right flank and left flank region (°C)</td>
</tr>
<tr>
<td></td>
<td>Lower right flank region SST (°C)-Mean ± SD</td>
<td>Lower left flank region SST (°C)-Mean ± SD</td>
<td>Difference in SST between right flank and left flank region (°C)</td>
</tr>
</tbody>
</table>

* p value < 0.001 between lower right flank region and lower left flank region, ** p value < 0.01 between lower right flank region and lower left flank region

SST distribution was symmetrical (difference in SST (°C) between right and left side was not significant) in the base line image before induction and also immediately after induction on the zeroth day in all the regions, and the value of asymmetrical SST (°C) difference percentage was < 0.5%. Then starting from the first week until ninth week after tumor induction asymmetrical SST (°C) distribution was observed (difference in SST (°C) was significant) in the lower flank region. The value of % asymmetrical SST (°C) difference in the tumor induced lower flank region was in the range of 0.5% to 2% whereas in abdomen and shoulder region it was < 0.5%.
3.3.4 Tumor development and SST (°C) variation

To see the progress of tumor development the thermogram ion images were converted into medical images, which assign different colors to different span of SSTs (°C) using FLIR software. Figure 3.4 represents infrared images generated from a single animal taken on different weeks after subcutaneous injection of DMBA carcinogen. The box in each image approximately (2×2) cm corresponds to the different region of split. Progress reduction in SST (°C) was observed, which can be identified by the changing of color from orange into red (Figure 3.4 a, b, c & d) in the tumor induced lower right flank region whereas in other regions symmetrically there was no color change.

3.3.5 Asymmetrical color histogram analysis

The color image was separated into different components as red, green and blue with different bins and the respective histogram was plotted using MATLAB program for all the six ROIs and compared symmetrically. Asymmetrical green component, i.e. the green pixel distribution was between 100 and 150 (Figure 3.5 a) in the lower-right flank region while in the lower left flank region, it was 175 to 200. Whereas the histogram of other abdomen and shoulder regions (Figure 3.5 c and d, e and f) was symmetrical, i.e. pixel density distributions were same in right and left regions. In the RGB Histogram analysis, asymmetry was observed only in the green components whereas in red and blue components, there was no predictable change. In a control Group-II, in all the regions (shoulder, abdomen and flank region) RGB color histogram was symmetrical as shown in Figure 3.6.

3.3.6 Biomarker - CEA (Carcino Embryonic Antigen) results

Serum CEA level measured using ELISA kit, in Table 3.2 also showed significant difference between mammary cancers induced group I and control group II. The mean tumor size was about 1.35cm in Group-I and the value of CEA level was 0.426 ±0.05 (ngm /ml). In control Group-II CEA level was 0.186 ±0.01 (ngm /ml).
Figure 3.2  Average SST (°C) in different regions: a). in the tumor induced lower flank region; b). in the abdomen region; c). in the shoulder region

Mean ± SD SST (°C) values (week wise) for 6 tumor induced rats from the specific ROIs of right and left side for all regions (Lower flank region, abdomen region and shoulder region) was plotted. There was a difference in SST (°C) observed between right and left flank regions. Tumor induced right side (lower right flank side) SST (°C) was less in all the weeks. There was no difference in SST (°C) observed between the right and left side of abdomen and shoulder regions.
Figure 3.3  Percentage asymmetrical difference in SST comparison between lower flanks (tumor induced region), abdomen and shoulder regions

(a)                                (b)                                (c)                                (d)

Figure 3.4  Whole body digital infrared pseudo color images of a single rat taken on different weeks (a. week 1, b. week 2, c. week 3, d. week 4) after tumor cancer induction by DMBA which shows the tumor growth by color change from thick orange (a), then light orange (b), then red color (c) and then to red color with pink center (d) in the fourth week indicating a reduction in SST (°C) progressively
Figure 3.5 RGB histogram of carcinogen induced single rat (Group–I) pseudo color thermal image in different ROIs: a).Lower right (tumor induced) flank region; b).Lower left flank region; c).Right abdomen region; d).Left abdomen region; e).Right shoulder region; f).Left shoulder region. Red and blue pixel distributions were symmetrical between right and left sides in tumor induced flank region except green pixel distribution (asymmetrical). All red, green and blue pixel distributions were symmetrical between corresponding right- and left- sides in non tumor sites (abdomen and shoulder regions).
Figure 3.6  RGB histogram of single rat (control Group–II) pseudo color thermal image in different ROIs: a).Lower right flank region; b).Lower left flank region; c).Right abdomen region; d).Left abdomen region; e).Right shoulder region; f). Left shoulder region. Red, blue and green pixel distributions were symmetrical between right and left sides in all the regions (flank, abdomen and shoulder).
Table 3.2 Serum CEA levels and tumor grading based on histopathology study

<table>
<thead>
<tr>
<th>Group</th>
<th>CEA level (ng/ml)</th>
<th>Mean tumor size (cm)</th>
<th>Tumor grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>I – Cancer induced group (n=6)</td>
<td>0.426 ±0.05</td>
<td>1.35</td>
<td>II – Ductal carcinoma (n=3)</td>
</tr>
<tr>
<td>II– Control (n=2)</td>
<td>0.186 ±0.01</td>
<td></td>
<td>IV– Lymph node with metastatic deposits (n=3)</td>
</tr>
</tbody>
</table>

Figure 3.7 a) Ductal carcinoma with arrow indicates ductal cells arranged in tubular pattern

Figure 3.7 b) Metastasis stage with arrow indicates lymph node with metastasis deposits.
3.3.7 **Histopathology results**

Histopathological results showed ductal carcinoma (grade III) in (n=3) rats in chemical carcinogen induced group and grade-IV metastases spread carcinoma in n=3 rats, and mean tumor size was about 1.35cm. Figure 3.7 a) was histopathology slides with different magnification of a rat with the infiltrating ductal carcinoma with size 1.5cm (comedo carcinoma) showing several layers of neoplastic cells and central necrosis of single rat mammary tissue. Arrow indicating typical ductal cells arranged in tubular pattern. Single lymph nodal with metastasis was present. Tumor showed ductular and cribriform pattern more than 50% ductal differentiation. In Figure 3.7 b), microscopical section of another rat’s mammary tissue showed lobules of breast parenchyma with attached skeletal muscle. Single lymph node included under this section showed metastatic deposits.

3.3.8 **Technical error of measurement (TEM) and coefficient of reliability (R)**

The values of average SST (°C) measurements made and the calculated values of TEM and R for all the above mentioned studies I to VI were tabulated in Appendix III of Tables A3.1 to A3.4. The Table A3.5 of Appendix III displayed the TEM and R values of all the above mentioned VI studies. From the table, it was found that the range of the values of TEM and R in study I were 0.04 to 0.06 and 0.990 to 0.994 respectively. Similarly the ranges of the values of TEM and R in study II were 0.04 to 0.07 and 0.991 to 0.994 respectively. The ranges of the values of TEM and R in study III were 0.03 to 0.15 and 0.980 to 0.994 respectively. The ranges of the values of TEM and R in study IV were 0.02 to 0.08 and 0.989 to 0.994 respectively. The ranges of the values of TEM and R in study V were 0.03 to 0.14 and 0.990 to 0.994 respectively and the same were found to be 0.03 to 0.14 and 0.988 to 0.994 respectively in study VI.

The ranges of the values of TEM and R in all the six studies (I to VI) were found to be 0.02 to 0.15 and 0.988 to 0.994 respectively. The measurement with the R value greater than 0.988 i.e., 98% of the variance was due to factors other than measurement error. TEM error also was found to be less than 0.15 i.e., 15% which confirms the reproducibility irrespective of the variation of average
measurements such as area, shape and position of ROI and also the measurements made at both different times and days.

3.4 DISCUSSIONS

In this study, whole-body thermal images of the rats with induced tumor were obtained dynamically without anesthesia in order to rule out heat loss associated with anesthetic drugs. Colman et al. (2002) reported a significant amount of heat loss in an anesthetized wistar rat. The decrease in SST (°C), compared to the base line measurement after 30 minutes of induction were found to be 1.75°C, 2.17°C and 1.9°C for the anesthetic drugs halothane, isoflurane and seveloflurane respectively [86]. In another study of 13762 MAT mammary adenocarcinoma induced rat model [80], it was observed that, when the rat was anesthetized using ether, the measured SST (°C) at the induced tumor site was lesser by 1°C than other non-tumor parts of rat.

The drop of skin temperature in the experimental animals, occurred between baseline readings and tumor induction. It may be due to the xenobiotics, and the endotoxin effects of the 7, 12-dimethyl benz (a)nthracene (DMBA), which was used to induce mammary tumors in wistar rats. It alters the behavioural and autonomic responses, which affects the thermoregulation of central nervous system, and results in hypothermia. Hence there was a decrease in skin surface temperature of wistar rats immediately after the injection of DMBA [75]. The another reason for drop of SST (°C) may be, as the injected medium DMBA was at room temperature (23.3°C) which was lesser than the body temperature of rats, immediately after injection produced a reduction in mouse skin temperature which lasted for about 3 hours [15].

In a xenograft induced breast tumor nude mice model using MDA-MB-231 and MCF7, the induced breast tumor had a lesser SST (°C) on 6th day of tumor induction, when comparing to non-tumor parts of the mice. It was reported that, the measured SSTs (°C) at the tumor site were lesser by 1.5 °C and 3 °C in MDA-MB-231 and MCF7 induced tumor mice models respectively [15]. In the present study, it was observed that, right side of the flank region, at which tumor was induced, had
lesser SST (°C) on 7th day of tumor induction, when comparing to base line measurements as well as other non-tumor parts of the rat. Further, during the third week after tumor induction, the measured mean SST (°C) at the tumor site (right flank region) was lesser by 3.8°C, when compared to non-tumor left flank region, which is agreed well with the xenograft induced mice models [15].

In a study of breast tumor in women using thermography, it was reported that, the measured SST (°C) of the tumor site was higher than the non-tumor part of the breast [87]. This finding was contradicted by the findings of breast tumor rat models, in which the measured SST (°C) was lesser at the induced tumor site than its normal counter parts from the body [15, 80] and also in our study.

In the present study, the baseline measurements (before tumor induction) showed that, the mean and SD value of SST (°C) at right flank side before tumor induction was 38.98±0.28. After tumor induction by the chemical carcinogens, the measured mean and SD of SST (°C) at the tumor induced right flank side was found to be decreased progressively, and it was 35.18±0.59 during third week after tumor induction. The reason for reduction in mean whole-body SST (°C) of the tumor bearing rat comparing to the baseline measurements may be due to the following: i).poor functioning of newly developed blood vessels; and ii).decreased blood flow at the tumor site by an autonomic nervous system [15, 80]. In the present study, the tumor induced right flank side of the rats showed a decrease in mean SST (°C) values throughout the study period of nine weeks, when compared to the corresponding non tumor left flank side. The measured asymmetrical SST difference at tumor bearing right flank sides of the rats was ranged from 0.5 to 2.0%, and it was statistically significant (p<0.001). On the other hand; the non-tumor regions (abdomen and shoulder) of the rats showed a decrease in mean SST (°C) values when compared to baseline measurements, but it was symmetrical in nature between the right and left side of the rats, and the measured SST difference was less than 0.5%.

Increase in SST (°C) was observed from fourth week. This may be due to metastasis condition, i.e. because of the spreading of cancer to other regions. This
increase was because of angiogenesis and higher metabolic rate of well developed cancerous cells.

From the first week to fourth weeks into the study, asymmetrical variation of SST (°C) was visually observed (by noting changes in colors of the thermogram) in all the rats (Figure.3.4 a, b, c and d). In the induced tumor site of the rat (right lower flank region), the SST (°C) was found to be decreased progressively from first to fourth weeks in the study. It was observed visibly by noting the color changed from orange in the first week (Figure 3.4 a) to light orange in the second week (Figure. 3.4 b), to red color in the third week (Figure 3.4 c) and to red with center pink color in the fourth week (Figure 3.4 d). On the other hand, in the non-tumor sites of the rat (abdomen and shoulder region), symmetrical nature of color variations were observed at right and left side of the rats. In human study, thermal images were analyzed for asymmetry between the patient’s breasts based upon different methods like Hurst coefficient [88], lacunarity coefficients [89] and by statistically texture analysis [90] to characterize the breast’s texture.

Thermal Texture Mapping (TTM) was found to be the superior method for detection of malignant neoplasm among the benign neoplasms than mammography and ultrasound images [91]. In the human breast tumor model, an increase in SST (°C) for the tumor region was demonstrated by a shift in the number of pixels from blue color to red color that can be calculated by either the number of pixels or by the percentage of pixel distribution in the image. A healthy woman seems to be fairly balanced between the three primary colors, while the woman with breast tumor appears to have a higher percentage of red color than blue color [92]. This higher percentage of red color may be an indication of the rise in SST (°C); on the other hand, the lower percentage of green color may be an indication of decrease in SST (°C). Kapoor et al (2010) proposed an automatic pattern classification, which clearly diagnosed the pathological changes of the breasts based on pixel distribution asymmetries [93]. The Red Green Blue (RGB) color histogram analysis of the thermal image is useful to study the asymmetric distribution of the SST (°C). In the tumor induced lower right flank region as well as in the non-tumor lower left flank region of the rat, red and blue color pixel distributions were same in the range of 230
to 255 and 0 to 10 respectively, whereas the green color pixel distribution was shifted to a lower level of 100 to 150 at the tumor induced right flank region than its corresponding value 175 to 200 measured at the non-tumor left flank region of the rat. The shifting of green pixel distribution clearly indicates a decrease in SST (°C) in the induced tumor site.

The non-tumor sites of abdomen and shoulder region of the rat showed symmetrical red, green and blue pixel distributions between right and left sides of the rats. The measured red, green and blue pixel distributions in the abdomen region of the rat were found to be 240 to 256, 175 to 220 and 0 to 10 respectively and these values were same in both right and left sides. Similarly in the shoulder region of the rat, the measured red, green and blue pixel distributions were found to be 240 to 256, 200 to 240 and 0 to 50 respectively, which were same in both right and left sides.

This study had few limitations. The number of rats used for this model was limited. Additional clinical and animal studies are required to prove whether thermography is useful in predicting the breast tumor well before. The finding from this study should be taken with the caution that it was related with small animal breast tumor model. Red, green and blue components used in this study were not the color spectrum intensity corresponds to the thermal scale. In future study, different color intensity labeled with the corresponding temperature range can be segmented and asymmetrical histogram analysis can be implemented. Some of the limitations of the SST (°C) measurement includes the following: i).There is possibility of error because of the artifacts due to movement of wistar rats while acquiring dynamic whole body thermal image without giving any anesthesia to the rats. But this was overcome by taking an average SST (°C) in the ROIs; ii).manual positioning of total number of six ROIs placed in the thermal image. There may be variation in the positioning of ROIs.

In conclusion, the findings from the study indicate that asymmetrical analysis of thermal images might have the considerable potential in monitoring progress of tumor and also diagnosis of cancer in early stage itself. Significant
(<0.01) difference in asymmetrical SST distribution was observed in tumor induced lower region whereas in other regions SST distribution was symmetrical. This was proven by asymmetrical pixel distribution in RGB Histogram. And also green component of the infrared image plays an important role in the asymmetrical histogram analysis for diagnosing a tumor in the animal model with decrease in SST (°C). Further this study can be extended for studying the response to anticancer drugs.