High Frequency Ultrasound Assessment of Skin Fibrosis: Clinical Results

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Running title:

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Abstract

Fibrosis is a common late effect of radiotherapy treatment for cancer patients. Current clinical assessment of radiation-induced fibrosis is generally limited to clinician-based rating scales, which are usually not sufficient for quantitative and objective evaluations. Ultrasonic propagation properties of tissues are widely reported to be sensitive to the alterations of tissue compositions and structures. Based on our previous feasibility study, we used four parameters including skin thickness and three ultrasonic parameters of dermal tissues (attenuation slope β, integrated attenuation IA and integrated backscatter IBS) in the frequency range of 10-25 MHz for the assessment of skin fibrosis. Experiments were conducted on the forearm and neck skin in patients with postirradiation fibrosis in the neck region. The palpation score and stiffness of the neck soft tissue were also measured as an indication of fibrotic severity. Comparisons of the results between 38 patients and 20 normal subjects showed a significantly smaller β (P = 0.005) and a significantly larger skin thickness (P < 0.004) and IA (P = 0.04) in the neck skins of the patients. However, age-matched comparisons showed there were neither significant differences among patient subgroups with different fibrotic levels assessed using manual palpation, nor significant correlations between the four parameters and the overall stiffness of the neck soft tissues (P > 0.05). In conclusion, ultrasound tissue characterization may provide additional information for the assessment of postirradiation skin fibrosis in the neck region. Further studies are necessary to investigate the feasibility of applying the current measurement for differentiating the severity of skin fibrosis. (Email: ypzheng@ieee.org)

Keywords: Radiotherapy, Fibrosis, Skin, Soft tissue, Nasopharyngeal carcinoma, NPC Attenuation, Backscatter, Hand palpation, Ultrasound indentation, Ultrasound

INTRODUCTION

Fibrosis is a common late side-effect of radiotherapy treatment for cancer patients and is considered to be a dose-limiting factor during the therapy. It has been reported that the latency of fibrosis is between 1 and 2 y postradiotherapy and the severity of fibrosis progresses over time (Bentzen et al. 1989). Radiation dose and fractionation schedule are believed to be related to the severity of fibrosis (Overgaard et al. 1987), though the underlying cellular processes of radiation-induced fibrosis are complex and not well understood (Rodemann and Bamerg 1995). Quantitative and objective assessment of fibrosis is helpful for oncologists and clinicians to estimate the efficacies of radiotherapy regimens or antifibrotic agents. For the quantification of radiation-induced fibrosis, biopsy examination of irradiated tissues is not preferred, due to its invasive nature. Clinically at present, the assessment of fibrosis is generally accomplished by clinician-based rating scales, which are often not sufficient for quantitative and objective evaluations (Davis et al. 2003). Skin is the most superficial tissue covering the human body so that it is more likely to develop late sequelae due to the direct penetration of irradiation beams (Porock et al. 1999). Therefore, it is necessary to develop some quantitative and noninvasive tools to provide diagnostically useful information for an *in-vivo* assessment of skin fibrosis.

Skin is a two-layered thin tissue in which the predominant dermis layer is mainly composed of elastic fibers and wavily interwoven collagen bundles in the ground substance interrupted by a network of blood and lymph vessels, nerves and appendages. Such a distinct structure of the dermis has made its ultrasonic observation very different from that of the subcutaneous fat tissues. High frequency ultrasound is capable of noninvasive, nonionizing and real-time examination of the skin with a relatively lower cost, in comparison with other procedures, such as biopsy and MRI. It has been successfully introduced in dermatology to detect skin structures and pathologies in the last two decades (Rallan and Harland 2003). Applications of ultrasound reported in dermatology are generally based on the measurements of skin thickness (Alexander and Miller 1979) and characteristics of ultrasonic images such as the echogenicity and textures (Seidenari et al. 1994). Such measurements have been applied to assess various skin conditions, such as natural ageing and skin diseases (De Rigal et al. 1989; Hoffmann et al. 1991). Among them, the skin sclerosis is particularly of interest to us, due to its involvement of a process similar to that of the fibrosis. Sclerosis is generally associated with an increase of fibrous collagen, which is a major structural protein in skin and composes approximately 75% of dry weight of skin (Hopewell 1990). Szymanska et al. (2000) reported that the sclerotic skin showed increased skin thickness and echogenicity in high frequency ultrasound images. Furthermore, it was reported that the skin plaques, measured by 15 MHz ultrasound, in patients with clinically “advanced” scleroderma were significantly thicker than those with “slight” scleroderma (Serup 1984). Gottlober et al. (1997) examined the cutaneous and subcutaneous tissues in patients who suffered from late effects of accidental radiation using ultrasound. Increased skin thickness was observed in their study. In extreme cases, the irradiated skin could be twice as thick as the contralateral nonirradiated counterpart. Based on their findings, they used the change of skin thickness as an indicator of cutaneous fibrosis in their later studies (Gottlober et al. 2001). Riekki et al. (2000) also reported a significant increase of thickness in breast skins after receiving radiotherapy using 20 MHz ultrasound. All these studies showed the potential use of the ultrasound detection of skin thickness in assessing the postirradiation reactions of the skins.

It has been widely reported that ultrasonic properties are sensitive to structural, compositional and pathological changes of acoustic scatterers in biological tissues (Thijssen 1989). Skin characterization using ultrasonic properties has been reported since the early 1980s for the assessment of various skin conditions, such as the wound *in vitro* (Olerud et al. 1987) and, recently, the skin dermatitis *in vivo* (Raju et al. 2003). It is potentially helpful to use the ultrasonic properties to characterize the irradiated skin fibrosis because some changes of the skin structures are induced by the therapeutic irradiation (Riekki et al. 2000). However, few clinical data were available to date in the literature for documenting the ultrasonic properties of fibrotic skins *in vivo*.

We have previously reported the high reliability of the measurement of ultrasonic properties of normal skin in the forearm and neck regions, including attenuation slope (β), integrated attenuation (IA) and integrated backscatter (IBS) by a 20 MHz ultrasound system with a hand-held probe (Huang et al. 2006). This measurement technique was applied clinically to 38 postradiotherapy patients in this study. Comparisons of the results were made between the 38 patients tested in this study and 20 normal subjects tested previously. Comparisons among the patient subgroups with different levels of fibrosis were also made. The measured parameters were correlated to the stiffness of neck soft tissues, which was regarded as another indicator of the severity of fibrosis. The tissue stiffness was measured using a tissue ultrasound palpation system (Zheng and Mak 1996, Zheng et al. 2000, Leung et al. 2002, http://tups.org).

MATERIALS AND METHODS

Ultrasonic data acquisition system

The data acquisition system used in the current study was the same as that reported in a previous study (Huang et al. 2006). It was briefly described in the present paper, and those who are interested in a detailed description can refer to that paper. The 20 MHz ultrasonic imaging system (Ultrasons Technologies, Tours, France) was mainly comprised of an ultrasonic unit, a personal computer and a cylindrical probe inside which there was a focused monoelement ultrasonic transducer (Fig. 1). The actual central frequency of the probe, measured in our lab, was 15 MHz with a -3 dB frequency bandwidth of 10-25 MHz. The ultrasound transducer was enclosed in a chamber and driven by a motor inside the probe. A solid cover with a thin membrane of 15 μm thickness was at the tip of the probe to form the chamber and contain the coupling media (water) between skin and transducer during operation. The transducer beam focused at approximately 2 mm beyond the membrane. The contact area of the probe with skin was small, with a dimension of 11×19 mm^2.

The system was capable of displaying the conventional B-mode images. At the same time, it could also collect backscattered radiofrequency (RF) signals, which were employed for extraction of ultrasonic parameters in the current study. In the RF-mode, the effective width in the lateral direction was approximately 6 mm, consisting of a set of 256 RF signal lines. The length of each line was 1024 points sampled by a 100 MHz A/D card. The lateral resolution of the transducer was approximately 0.2 mm, thus producing a total of 28 independent lines for each set of the 256 lines per image (Huang et al. 2006). These 28 independent lines were used in the spatial averaging for the extraction of the ultrasonic parameters. Water was used as the coupling medium in all the experiments.

Subjects and experiments

The clinical studies were performed in the Department of Clinical Oncology, Prince of Wales Hospital, Hong Kong, between June and September, 2004. Totally, 38 patients (age: 54 ± 11 y; range: 36-76 y), who had received therapeutic irradiation to the full length of both sides of the neck in treatment of nasopharyngeal carcinomas, were recruited in their clinical follow-up for the study. The median of the follow-up time was 3.0 y, ranging from 1.0 to 19.0 y. This study was approved by the Research Ethics Committee of the investigators' affiliated institutions and written informed consent was obtained from each subject at recruitment.

The test regions were selected and marked in the palmar side of the distal forearm (3 cm away from the radiocarpal joint and along the media nerve) and in both sides of the neck (5 cm below the mastoid bone). The forearm test site served as the control site to adjust for the effects not related to radiotherapy, such as ageing. We had previously selected both the palmar and dorsal skin of the forearm as the control sites in the tests on normal subjects (Huang et al. 2006). In this study, the dorsal skin was neglected for simplicity, because no significant difference between the two sides of the forearm in normal subjects has been found (Huang et al. 2006). For the ease of probe operation, subjects were seated when the forearm sites were tested and lay on their sides when the neck sites were tested. Before data collection, the probe's orientation was adjusted to be perpendicular to the skin surface and to assure a good coupling between the probe and skin during data collection. For each testing site, three repeated tests were conducted and the results were averaged to represent the mean value for that testing site.

An ultrasound indentation test was also conducted at the same site of the neck tissues after the RF signals were collected for each patient. The indentation test was accomplished using a minimized version of the tissue ultrasound palpation system (Leung et al. 2002). The detailed operation of this system was described in the previous publications (Zheng and Mak 1996, Zheng et al. 2000, Leung et al. 2002). The load-deformation data obtained by the ultrasound indentation were used to calculate the stiffness of the neck soft tissue. Typically, three indentation tests were performed for each side of the neck and the results were averaged for each testing site. The whole experiment, including the measurements using the 20 MHz ultrasound imaging system and the ultrasound indentation system, was completed in approximately 30 min for each subject.

Skin thickness and ultrasonic parameters

In this study, the skin thickness was measured from the RF images. In the image, the dermis-fat interface could be clearly observed, while the epidermis-dermis interface was not differentiable using the current probe (Fig. 2). Thus, the skin thickness, which is the sum of epidermis and dermis, could be obtained by using the points between the skin entry and dermis-fat interface echoes based on the assumption of a constant speed of sound of 1.58 mm/μs throughout the whole skin layer (Alexander and Miller 1979). The measurement of thickness was performed at the selected region in the image, which contained a distinct dermis-fat interface for manual segmentation. The selected region should occupy at least half of the full image (Fig. 2). The distances between the upper and lower borders of the selected region were averaged as the mean skin thickness.

The ultrasonic propagation parameters were extracted from the RF signals using a multinarrowband algorithm (Bridal et al. 1997). Reference signals from a planar steel plate were also collected in order to compensate the system-dependent effects (Fournier et al. 2001). The studied region of interest of the skin was approximately from 0.3 to 1.3 mm beneath the skin surface. As epidermis and dermis were not resolvable on the ultrasound image, we didn't differentiate them when the ultrasonic parameters were extracted. However, we had disposed the high reflection skin entry echo region in order not to skew our results by including a highly inhomogeneous region. The detailed procedure for the extraction of these parameters could be found in the previous paper (Huang et al. 2006). A brief description was given as follow.

Attenuation slope (β) and integrated attenuation (IA). Assume that a linear frequency-dependent $\alpha(f)$ of the skin was extracted in the frequency range of 10 to 25 MHz and written as:

$$\alpha(f) = \beta \cdot f + \alpha_0 \quad (1)$$

where β (unit: dB/mm/MHz) is the attenuation slope and α_0 (unit: dB/mm) is the intercept of the attenuation coefficient at the zero frequency. β was then calculated from the regression of $\alpha(f)$ to f at discrete frequency points. The integrated attenuation (IA, unit: dB/mm) was defined as:

$$\mathbf{IA} = \frac{\mathbf{1}}{f_h - f_l}\int_{f_l}^{f_h} \alpha(f)df \quad (2)$$

where $f_l = 10$ MHz and $f_h = 25$ MHz were -3 dB bandwidth frequencies of the transducer.

Integrated backscatter (IBS). The backscatter spectra $B(f)$ of the skin were extracted after the system-dependent effect of the measurement was corrected. Thus, integrated backscatter (IBS, unit: dB), which represented the average level of backscatter of the skin, was defined in the similar way as that of IA:

$$\mathbf{IBS} = \frac{1}{f_h - f_l}\int_{f_l}^{f_h} B(f)df\ . \tag{3}$$

A custom-designed program written in Matlab (MathWorks, Natick MA, USA) was used to compute the ultrasonic parameters.

Evaluations of severity of fibrosis

Hand palpation. During his/her follow-up in the Department of Clinical Oncology, each patient was palpated by an experienced oncologist and a palpation score ranging from 0 to 3 was given to indicate the severity of fibrosis for the patient. The scoring criteria were explained as follows: grade 0, nil or equivocal presence of fibrosis; grade 1, unequivocal presence of fibrosis of mild degree; grade 2, moderately severe fibrosis change; and grade 3, severe fibrosis. The oncologist who rated the patients was blind to the results of the ultrasonic measurement. According to the palpation score, the patients were further divided into four subgroups, as indicated by group 0 to group 3 for subsequent data analyses. The demographic information of the patients is shown in Table 1.

Neck soft tissue stiffness. Effective Young's modulus (YM) of the neck soft tissue was measured as another indicator of severity of the fibrosis (Leung et al. 2002) using the ultrasound indentation method (Zheng and Mak 1996, Zheng et al. 2000).

Statistics

The skin thickness and ultrasonic parameters were compared between the neck and forearm skin of the patients using the paired t-test. The results of the neck tissues were also compared between the patients and the 20 normal subjects (age: 27 ± 3 y; range: 23-32 y) using the unpaired t-test. The normal subjects had been tested previously in the same way as in the current study (Huang et al. 2006). The ultrasonic parameters of the forearm skin were also correlated to the subject age. If age-dependence of a parameter was found, an extra analysis would be made for its differential representation, i.e., the parameter of the neck site subtracted by that of the control site (denoted by a subscript "d"). It was assumed that the comparison based on the differential representation of the parameter might be more meaningful, as it was less age-dependent. According to Table 1, the patient subgroups were assumed to be age-matched. Thus, comparison was also made among patient subgroups using the nonparametric one-way ANOVA (Kruskal-Wallis test), considering a relatively small number of samples for each group. The four parameters were also correlated to the stiffness of neck soft tissues to see whether there were any correlations between the parameters and severity of fibrosis. The statistics toolbox in Matlab was used to perform all the statistical analyses and $P < 0.05$ was used as a significance level.

RESULTS

Skin thickness and ultrasonic parameters in patients

The interface between the dermis and fat was generally observable in both the forearm skin and the irradiated neck skin in the patients (Fig. 2). Figure 3 shows the skin thickness measured at the three test sites in the patients. It was 1.52 ± 0.24 mm in the forearm. For the left and right sides of the neck, the skin thickness was 2.06 ± 0.24 mm and 2.18 ± 0.35 mm, respectively ($P < 0.001$). The skin in both sides of the neck was significantly thicker than that in the forearm ($P < 0.001$).

Figure 4 shows the ultrasonic parameters measured in the patients. For all the three parameters, no significant difference was found for the skins in the left and right sides of the neck ($P > 0.05$). Thus, for each of the parameters, the results of the left and right sides of the neck were pooled to obtain an averaged value, which was used to compare with that of the forearm. β was 0.303 ± 0.034 and 0.368 ± 0.048 dB/mm/MHz, IA was 2.85 ± 1.93 and 1.03 ± 1.76 dB/mm and IBS -34.00 ± 2.29 and -29.77 ± 2.66 dB for the neck and forearm skins, respectively. In comparison with the forearm skin, β ($P < 0.001$) and IBS ($P < 0.001$) of the neck skin were significantly smaller and IA ($P < 0.001$) was significantly larger.

Comparisons between normal subjects and patients

Figure 3 shows the comparison of the skin thickness between the patients and the normal subjects. The skin thickness of the forearm was not significantly different between the two groups ($P > 0.05$), while it was significantly larger for the left (2.06 ± 0.34 vs. 1.82 ± 0.27 mm, $P = 0.004$) and right (2.18 ± 0.35 vs. 1.88 ± 0.36 mm, $P = 0.002$) sides of the neck of the patients in comparison with the normal subjects.

Figure 4 shows the comparisons of the ultrasonic parameters between the patients and the normal subjects. Direct comparison of the neck skin between the two groups showed that β of the patients was significantly smaller (0.303 ± 0.034 vs. 0.328 ± 0.029 dB/mm/MHz, $P = 0.005$), IA was significantly larger (2.85 ± 1.93 d vs. 1.93 ± 1.81 dB/mm, $P = 0.04$), and IBS was slightly smaller (-34.00 ± 2.29 vs. -33.55 ± 2.98 dB, $P = 0.26$) than those of the normal subjects.

When the four parameters were correlated to the subject age in the forearm skin of the normal subjects and patients, a significant correlation between IBS ($P < 0.05$) and age was found, while this was not the case for the other three parameters (Table 2). Hence, a comparison of the relative representation of IBS, as denoted by IBS_d, was further performed. IBS_d was significantly smaller (–4.23 ± 2.59 vs. –1.98 ± 2.13 dB, $P < 0.001$) in the patients.

Correlations with two measurements of fibrosis

For each patient subgroup, which was formed on the basis of the degree of fibrosis evaluated by the oncologist, the four parameters were calculated (Table 3) and compared directly according to the matched age distribution (Table 1). Kruskal-Wallis test showed no significant difference for the means of the four parameters among the four subgroups ($P > 0.05$).

For the measurement of the neck soft tissue stiffness, the indentation test was successfully conducted for totally 33 patients (it failed for five patients, due to the malfunction of the testing system during the experiment). The measured effective YM was found to be significantly different among the four patient subgroups ($P < 0.03$), and it increased with the increase of the palpation score (Table 1). However, all the correlations between the four parameters and the effective YM for both sides of the neck in the 33 patients did not reach a significance level ($P > 0.05$).

DISCUSSION

To the best of the authors' knowledge, little literature exists to document the ultrasonic propagation properties in skins *in vivo* for patients with radiation-induced fibrosis. The present study attempted to provide some basic clinical results for this purpose, with the aim to achieve a better assessment of skin fibrosis. The results demonstrated some significant differences of the thickness and ultrasonic parameters between the skin with fibrosis in patients and those of the normal subjects. These differences were regarded to be caused by the fibrotic process. However, the severity levels of the fibrosis could not be successfully differentiated, based on the results obtained in the current study.

Fibrotic skin thickness

The accuracy of the ultrasonic measurement of skin thickness has been established since the late 1970s (Alexander and Miller 1979). By introducing this method in the current study, it was found that the neck skin thickness increased significantly in the patients after radiotherapy, which was consistent with previous publications (Gottlober et al. 1997; Riekki et al. 2000; Warszawski et al. 1997). In the present study, skin thickness of the patients was approximately 13% to 16% (left side: 2.06/1.82 mm; right side: 2.18/1.88 mm) larger than that of the normal subjects, which was similar as that reported by Riekki et al. (2000) (1.84/1.62 mm). However, the increase was generally smaller than those reported by Gottlober et al. (1997) and Warszawski et al. (1997), who reported increases of 37% (2.28/1.66) in the neck region and 38% (2.31/1.68) in the breast skin, respectively. One possible reason for the discrepancies was the difference of radiation dosage used for the patients. Another possible reason was the difference of the follow-up time of the subjects at recruitment. Thus, to document the intensity of radiation and to record the time of follow-up are important for reporting and comparing the change of skin thickness after radiation.

Ultrasonic parameters of fibrotic skins

We had previously reported that the measurement of the ultrasonic properties using the present ultrasonic system was highly reliable (Huang et al. 2006). The intra- and inter-rater measurement was demonstrated to be highly reliable as indicated by ICC values generally larger than 0.80. Thus, clinical experiments were performed to evaluate its capability in assessing the tissue fibrosis. The results of the current study showed that β decreased and IA increased significantly in the neck fibrotic skin. This might indicate an increase of the total attenuation but with a decreased attenuation slope. Bridal et al. (1997) also reported a decrease of β and an increase of IA in their study of atherosclerotic plaques in the frequency range of 30 to 50 MHz. The results showed that IBS slightly decreased in the neck fibrotic skin, which was consistent with what we observed in the current experiments, that the image brightness was generally lower in the patients than that in the normal subjects, noting that IBS defined in this study was comparable with the echogenicity as in an ultrasonic image. Our findings of IBS were consistent with those reported by Warszawski et al. (1997) that a rapid decrease of the echogenicity was detectable in the early reaction after radiotherapy and then it increased slightly but was still less than the normal value at the time defined as the late reaction in their study.

It was noted that there was a significant correlation between IBS and age for the intact forearm skin of the normal subjects and patients. It was reasonable to assume that aging might have a similar effect to the neck skin. To reduce the age effect on the comparison of IBS between the patients and the normal subjects, we used the differential representation of IBS, which was defined as the subtraction of IBS of the neck skin and that of the forearm skin of each subject and denoted as $\mathrm{IBS_d}$. The results showed that $\mathrm{IBS_d}$ was significantly smaller in the patients. Our findings suggested that the aging effects on the measurement of IBS should be carefully considered in the similar studies in the future, when using age-matched normal and patient groups.

Correlations of four parameters and severity of soft tissue fibrosis

Hand palpation is a well-accepted clinician-based measure for fibrotic severity (Davis et al. 2003). The ultrasound indentation method to measure the tissue stiffness had been demonstrated to be quite reliable for the assessment of tissue fibrosis (Zheng et al. 2000). Thus, they have been introduced in this study to provide a measure of the fibrotic severity for comparisons with other parameters. The quantitative measurement of stiffness of the neck soft tissue obtained using the ultrasound indentation test were well correlated with the palpation score, which agreed well with the results reported in a previous study (Leung et al. 2002). No obvious conclusions on the relationship between the degree of neck tissue fibrosis and the four parameters could be drawn from the current results. However, it should be noted, in the current study, that the degree of the skin fibrosis was reflected either by the hand palpation scale of the neck soft tissue given by a clinical oncologist, or by the measured stiffness value, both of which indicated the mechanical properties of the entire neck soft tissues, i.e., the skin as well as the subcutaneous tissues. Thus, the obtained severity level of the skin fibrosis was very likely to have some bias with respect to the true state of the skin. It warrants a specific measure of the mechanical properties of the skin tissue in future investigations in order to correlate exactly the mechanical properties of the skin to the studied parameters.

Possible reasons for changes of four parameters in irradiated skins

The water content and collagen of the skin might serve as candidate components responsible for the changes of the measured parameters. The interchangeable water forms 1/3 of the extracellular matrix and the redistribution of the water was demonstrated to alter the skin thickness (Eisenbeiss et al. 2001). The skin thickness increased when more water was stored in the skin. The water content also had negative effects on the ultrasonic attenuation (Olerud et al. 1987). An increase of water content would cause a decrease of the echogenicity (Gniadecka and Quistorff 1996). The water content of the irradiated skin during and after radiation was reported to have two phases of change. Nuutinen et al. (1998) reported that the water content decreased during the time of radiation treatment, due to the radiation-induced damage to the skin capillaries. But it tended to increase, due to the increase of water-bound collagen and proteoglycan content, 2 years after the treatment. The other component which might induce the changes of the parameters is collagen. It is a main element of the dry weight of the skin and apparently contributes to the alteration of skin thickness in a fibrotic process (Rodnan et al. 1979). It exists in the skin in the form of interwoven collagen fibers, so that the orientation of the collagen fibers serves as another source of variation of the ultrasonic properties, especially for backscatter (Roberjot et al. 1997). It was also reported that the collagen had a positive correlation with attenuation and backscatter coefficients (Moran et al. 1995; Olerud et al. 1987). There were evidences that the collagen changed significantly both in concentration and structure in fibrotic skin (Leontiou et al. 1993; Riekki et al. 2000). In general, changes of the measured parameters in the fibrotic skin might be related to the alterations of both the elements. Based on the results of the current study, one reasonable explanation was that IA might be more sensitive to the increase of collagen, whereas IBS might be more sensitive to the increase of water. That is to say, the increase of IA might mainly result from the increased collagen concentration and the decrease of IBS, mainly from more storage of water content. Variations of β might be caused by a change of microstructure of the fibrils in the fibrotic skin, as β is very dependent on ultrasonic scatterers. As the change of the water and collagen content was not quantified in the current study, the validity of such a hypothesis needs to be further confirmed in future studies.

CONCLUSIONS

The skin thickness and the ultrasonic properties of the neck skin with fibrosis in patients were measured and compared with those of the normal subjects. It was demonstrated that the skin thickness and IA increased, while β and IBS decreased in the fibrotic skin tissues. No significant correlation between the four parameters and the severity of neck soft tissue fibrosis was found. Possible reasons for the changes of the studied parameters in the fibrotic skins were discussed. Alterations of water and collagen contents might be responsible for the changes of measured parameters. Further studies are needed to confirm this explanation by quantifying the changes of the water and collagen in fibrotic skins. Two directions may be considered in future investigations to study how the severity of fibrosis affects the skin thickness and ultrasonic parameters. One is to conduct biochemical or histological examinations directly to quantify the level of skin fibrosis. And the other is specifically to measure the physical properties, such as the elasticity of the skin layer, as an indicator of the cutaneous fibrosis. A more direct way to study the effect of radiation-induced fibrosis is to conduct parameter comparisons before and after the therapeutic radiation. In such a way, longitudinal monitoring of the change of the various parameters will also be possible using the methods proposed in the current study.

Acknowledgements - This work was supported by the Research Grant Council of Hong Kong (PolyU 5245/03E) and the Hong Kong Polytechnic University. Sincere appreciations were given to Dr. Chen Xin, Mr. Huang Qinghua, Miss. Lu Minghua and Mr. Chen Jie for their help in conducting some parts of the experiments and during the preparation of manuscript of this paper. The authors also thanked the anonymous reviewers for their good comments that had improved the quality of the paper.

REFERENCES

Alexander H, Miller DL. Determining skin thickness with pulsed ultra sound. J Invest Dermatol 1979;72:17-19.

Bentzen SM, Thames HD, Overgaard M. Laten-time estimation for late cutaneous and subcutaneous radiation reactions in a single-follow-up clinical study. Radiother Oncol 1989;15:267-274.

Bridal SL, Fornes P, Bruneval P, Berger G. Correlation of ultrasonic attenuation (30 to 50 MHz) and constitutes of atherosclerotic plaque. Ultrasound Med Biol 1997;23:691-703.

Davis AM, Dische S, Gerber L, et al. Measuring postirradiation subcutaneous soft-tissue fibrosis: state-of-the-art and future directions. Semin Radiat Oncol 2003;13:203-213.

De Rigal J, Escoffier C, Querleux B, et al. Assessment of aging of the human skin by in vivo ultrasonic imaging. J Invest Dermatol 1989;93:621-625.

Eisenbeiss C, Welzel J, Eichler W, Klotz K. Influence of body water distribution on skin thickness: measurements using high-frequency ultrasound. Br J Dermatol 2001;144:947-951.

Fournier C, Bridal SL, Berger G, Laugier P. Reproducibility of skin characterization with backscattered spectra (12-25 MHz) in healthy subjects. Ultrasound Med Biol 2001;27:603-610.

Gniadecka M, Quistorff B. Assessment of dermal water by high-frequency ultrasound: comparative studies with nuclear magnetic resonance. Br J Dermatol 1996;135:218-224.

Gottlober P, Kerscher MJ, Korting HC, Peter RU. Sonographic determination of cutaneous and subcutaneous fibrosis after accidental exposure to ionising radiation in the course of the Chernobyl nuclear power plant accident. Ultrasound Med Biol 1997;23:9-13.

Gottlober P, Steinert M, Bahren W, et al. Interferon-gamma in 5 patients with cutaneous radiation syndrome after radiation therapy. Int J Radiat Oncol Biol Phys 2001;50:159-166.

Hoffmann J, Gerbaulet U, El-Gammal S, Altmeyer P. 20-MHz B-mode ultrasound in monitoring the course of localized scleroderma (morphoea). Acta Dermatol Venereol 1991;164:S3-16.

Hopewell JW. The skin: its structure and response to ionizing radiation. Int J Radiat Biol 1990;57:751-773.

Huang YP, Zheng YP, Leung SH, Mak AFT. Reliability of measurement of skin ultrasonic properties in vivo: a potential technique for assessing irradiated skin. Skin Res Tech, 2006, in print.

Leontiou I, Matthopoulos DP, Tzaphlidou M, Glaros D. The effect of gamma irradiation on collagen fibril structure. Micron 1993;24:13-16.

Leung SF, Zheng YP, Choi CYK, et al. Quantitative measurement of post-irradiation neck fibrosis based on the Young modulus: description of a new method and clinical results. Cancer 2002;95:656-662.

Moran CM, Bush NL, Bamber JC. Ultrasonic propagation properties of excised human skin. Ultrasound Med Biol 1995;21:1177-1190.

Nuutinen J, Lahtinen T, Turunen M et al. A dielectric method for measuring early and late reactions in irradiated human skin. Radiother Oncol 1998;47:249-254.

Olerud JE, O'Brien W Jr., Riederer-Henderson MA, Steiger D, Forster FK, Daly C, Ketterer DJ, Odland GF. Ultrasonic assessment of skin and wounds with the scanning laser acoustic microscope. J Invest Dermatol 1987;88:615-623.

Overgaard M, Bentzen SM, Christensen JJ, et al. The value of the NSD formula in equation of acute and late radiation complications in normal tissue following 2 and 5 fractions per week in breast cancer patients treated with postmastectomy irradiation. Radiother Oncol 1987;9: 1-11.

Porock D, Nikoletti S, Kristjanson L. Management of radiation skin reactions: literature review and clinical application. Plast Surg Nurs 1999;19:185-192.

Raju B, Swindells KJ, Gonzales S, Srinivasan MA. Quantitative ultrasonic methods for characterization of skin lesions in vivo. Ultrasound Med Biol 2003;29:825-833.

Rallan D, Harland CC. Ultrasound in dermatology-basic principles and applications. Exp Dermatol 2003;28:632-638.

Riekki R, Jukkola A, Sassi ML, et al. Modulation of skin collagen metabolism by irradiation: collagen synthesis is increased in irradiated human skin. Br J Dermatol 2000;142:874-880.

Roberjot V, Laugier P, Berger G. Anisotropy in bovine skeletal muscle *in vitro*: frequency dependent attenuation and backscatter coefficient over a wide range of frequencies. IEEE Ultrasonic Symposium Proceedings, 1994:1467-1470.

Rodemann HP, Bamerg M. Cellular basis of radiation-induced fibrosis. Radiother Oncol 1995;35: 83-90.

Rodnan GP, Lipinski E, Luksick J. Skin thickness and collagen content in progressive systemic sclerosis and localized scleroderma. Arthritis Rheum 1979;22:130-140.

Seidenari S, Pagnoni A, Di Nardo A, Giannetti A. Echographic evaluation with image analysis of normal skin: variations according to age and sex. Skin Pharmacol 1994;7:201-209.

Serup J. Localized scleroderma (morphoea): thickness of sclerotic plaques as measured by 15 MHz pulsed ultrasound. Acta Dermatol Venereol 1984;64:214-219.

Szymanska E, Nowicki A, Mlosek K, et al. Skin imaging with high frequency ultrasound – preliminary results. Eur J Ultrasound 2000;12:9-16.

Thijssen JM. Ultrasonic tissue characterisation and echographic imaging. Phys Med Biol 1989;34:1667–1674.

Warszawski A, Rottinger EM, Vogel R, Warszawski N. 20 MHz ultrasonic imaging for quantitative assessment and documentation of early and late postradiation skin reactions in breast cancer patients. Radiother Oncol 1997;47:241-247.

Zheng YP, Mak AFT. An Ultrasound indentation system for biomechanical properties assessment of soft tissues in-vivo. IEEE Trans Biomed Eng 1996;43:912-918.

Zheng YP, Leung SF, Mak AFT. Assessment of neck tissue fibrosis using an ultrasound palpation system: A feasibility study. Med Biol Eng Comp 2000;38:1-6.

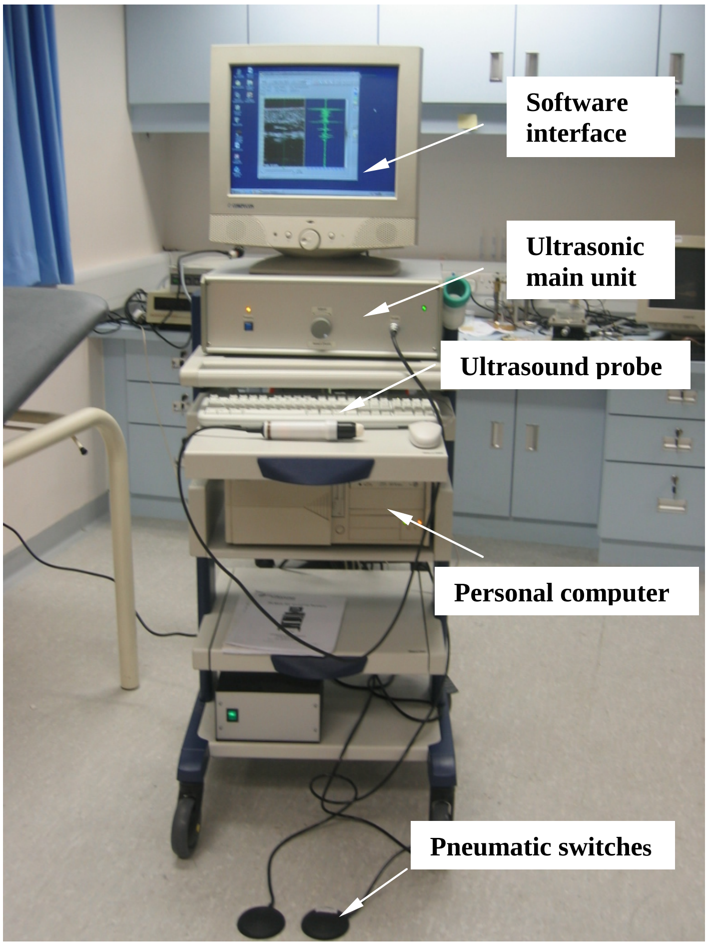
Figure captions

Fig 1. (a) The high frequency (20 MHz) ultrasonic imaging system; (b) Zoomed software interface; (c) Operation of the ultrasonic probe.

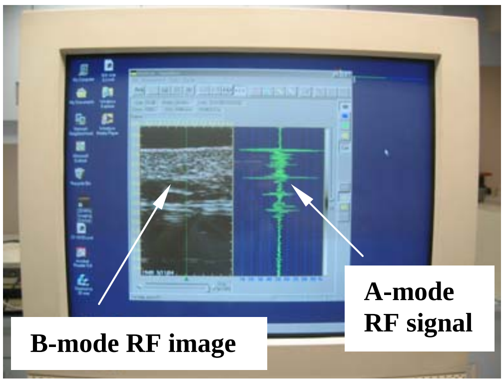
Fig. 2. Typical RF B-mode images of the skin for the (a) Forearm and (b) Neck regions in a patient with fibrosis. The skin thickness measured was 1.38 mm and 1.83 mm for (a) and (b), respectively. The white bar in the left-right corner indicates 1 mm. The horizontal dashed line was marked for the measurement of the skin thickness.

Fig. 3. Skin thickness at various test sites of the normal subjects and patients.

Fig. 4. Ultrasonic parameters of skin in the forearm and neck regions of the normal subjects and patients: (a) Attenuation slope (beta); (b) Integrated attenuation (IA); (c) Integrated backscatter (IBS).


Software
interface
Ultrasonic
main unit
Ultrasound probe
Personal computer
Pneumatic switches

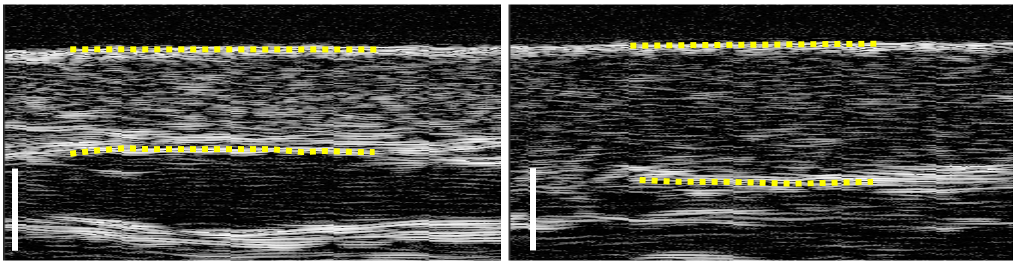
(a)


A-mode
RF signal
B-mode RF image

(b)

(c)

Fig. 1



(a) (b)

Fig. 2.

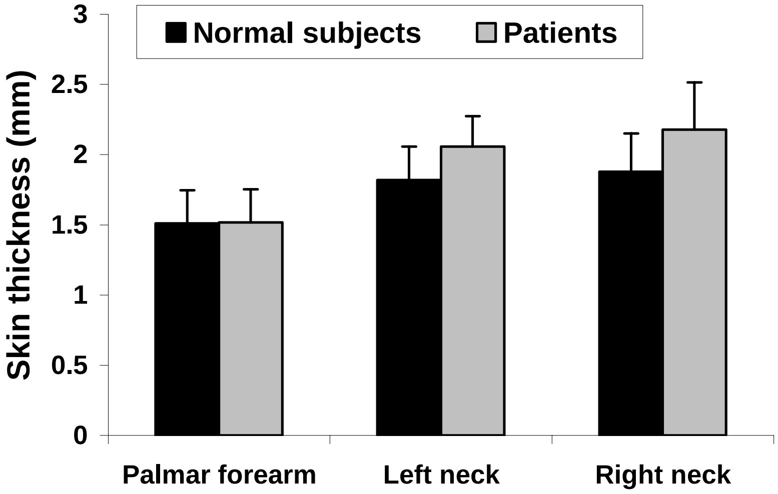

Normal subjects
Patients
Skin thickness (mm)
3
2.5
2
1.5
1
0.5
0
Palmar forearm
Left neck
Right neck

Fig. 3.

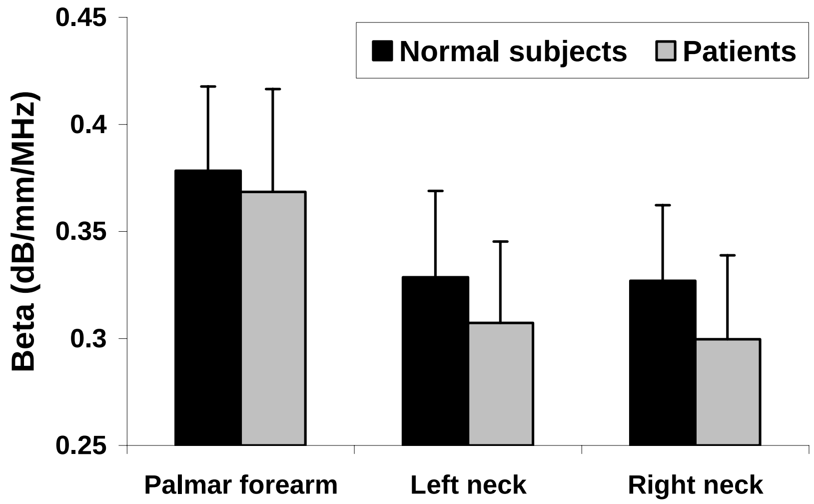


Fig. 4.a

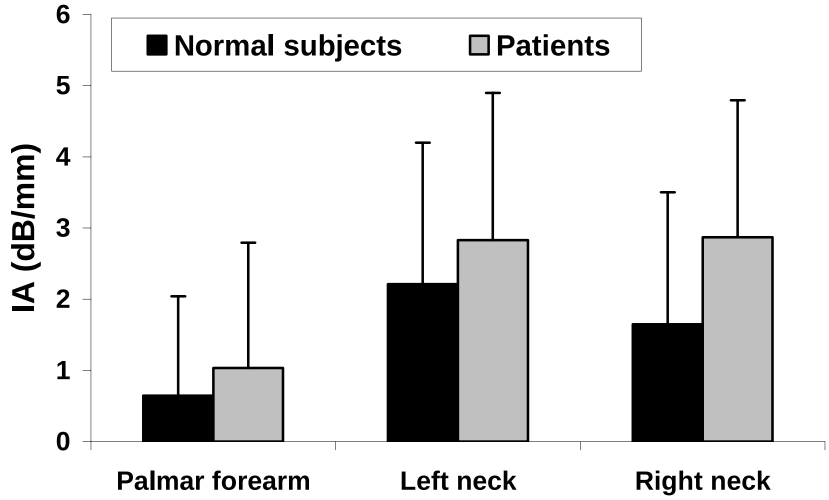


Fig. 4.b

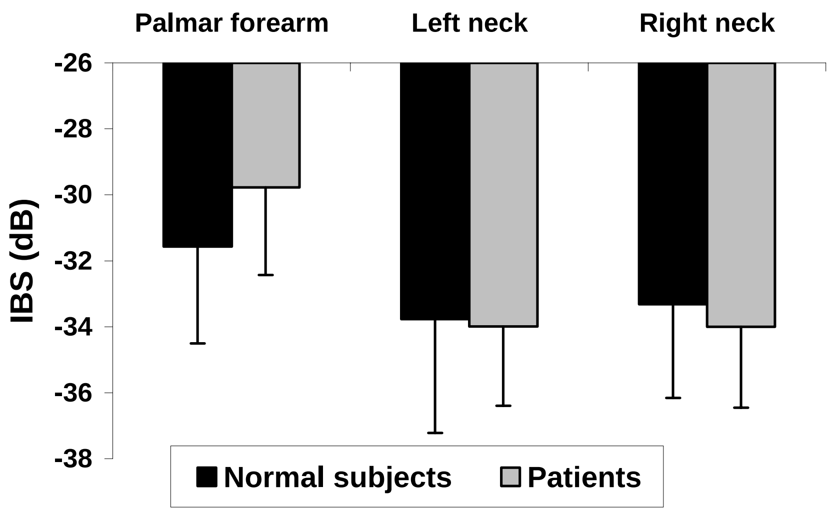


Fig. 4.c

Table 1. The demographic information and measured stiffness of the neck soft tissues for the four patient subgroups

Group	Population (*n*)	Mean age (y) (SD)	Effective YM* (kPa)
0	13	54 (10)	54 (16)
1	11	54 (13)	66 (11)
2	8	57 (12)	86 (17)
3	6	53 (10)	129 (36)

*: the actual number of subjects for whom the effective YM was successfully measured was 10, 10, 7, 6 for group 0 to group 3.

Table 2. Correlations between the four parameters of the forearm skin and the subject age (including all the patients and normal subjects).

Correlations	Skin thickness and age	β and age	IA and age	IBS and age
r	-0.001	-0.12	0.15	0.33*

*: $P < 0.05$

Table 3. Mean and standard deviations of the skin thickness and ultrasonic parameters of the irradiated neck skin among four patient subgroups.

Patients	Group 0	Group 1	Group 2	Group 3	Overall
Skin thickness(mm)	2.07 (0.34)	2.18 (0.32)	2.16 (0.42)	2.05 (0.20)	2.12 (0.33)
β (dB/mm/MHz)	0.309 (0.035)	0.294 (0.036)	0.299 (0.042)	0.316 (0.019)	0.303 (0.034)
IA (dB/mm)	3.24 (1.91)	3.01 (2.23)	2.11 (1.90)	2.70 (1.60)	2.85 (1.93)
IBS (dB)	-34.20 (1.59)	-34.35 (2.51)	-33.77 (3.03)	-33.22 (2.43)	-34.00 (2.29)