Acoustically Accessible Window Determination for Ultrasound Mediated Treatment of Glycogen Storage Disease Type Ia Patients

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\textbf{Abstract.} Glycogen storage disease type Ia (GSDIa) is caused by an inherited single-gene defect resulting in an impaired glycogen to glucose conversion pathway. Targeted ultrasound mediated delivery (USMD) of plasmid DNA (pDNA) to liver in conjunction with microbubbles may provide a potential treatment for GSDIa patients. As the success of USMD treatments is largely dependent on the accessibility of the targeted tissue by the focused ultrasound beam, this study presents a quantitative approach to determine the acoustically accessible liver volume in GSDIa patients. Models of focused ultrasound beam profiles for transducers of varying aperture and focal lengths were applied to abdomen models reconstructed from suitable CT and MRI images. Transducer manipulations (simulating USMD treatment procedures) were implemented via transducer translations and rotations with the intent of targeting and exposing the entire liver to ultrasound. Results indicate that acoustically accessible liver volumes can be as large as 50\% of the entire liver volume for GSDIa patients and on average 3 times larger compared to a healthy adult group due to GSDIa patients’ increased liver size. Detailed descriptions of the evaluation algorithm, transducer- and abdomen models are presented, together with implications for USMD treatments of GSDIa patients and transducer designs for USMD applications.

\textbf{Keywords:} acoustic accessibility, GSDIa, targeted delivery, pDNA, G6Pase.

\section*{INTRODUCTION}

Glycogen storage diseases (GSD), affecting approximately 1 in 100,000 births in the United States \cite{1}, are caused by various genetic defects that impair the glycogen breakdown pathway. Young patients, particularly infants, are frequently at high risk of life threatening acute hypoglycemia, which could potentially lead to seizures, coma and even death. This study focuses on glycogen storage disease type Ia (GSDIa), in which a glucose-6-phosphatase (G6Pase) gene defect prevents the synthesis of the G6Pase enzyme. Excessive glycogen can’t be converted into glucose in the absence of the G6Pase enzyme, and accumulated glycogen leads to an enlarged liver in most GSDIa patients. Up to now, no cure for GSDIa patients is available, and strict dietary control using cornstarch has been the principle method to manage this disease for the past 29 years. With the development of gene therapy, a potential treatment for GSDIa patients has been proposed recently. By introducing a non-defective G6Pase gene (in
the form of a plasmid) to liver cells, G6Pase enzyme production may be restored. Focused ultrasound (FUS) in combination with microbubbles has been proposed to deliver pDNA into liver cells via sonoporation and other mechanisms [2]. It has been shown that this approach has the effect of increasing the permeability of blood vessels and cell walls, leading to enhanced uptake of treated liver cells. Furthermore, the injected microbubbles can also be used as a contrast agent for ultrasound imaging, providing real-time guidance for the procedure.

In order for the USMD procedure to be effective, sufficient liver cells need to be transfected with pDNA. Liver tissue that is partially hidden inside the rib cage typically cannot be easily accessed by the ultrasound beam due to the limited acoustic window. Thus, it is necessary to estimate what liver volume will be accessible by FUS, and which transducer geometry and arrangements are best suited for such an application. In this study, we investigated these questions quantitatively via a geometric algorithm combined with acoustic field simulations, utilizing 3D image datasets from 5 GSDIa patients (MRI or CT scans). The same algorithm was then also applied to estimate the acoustically accessible liver volume of 3 healthy adult subjects for comparison purposes.

METHODS

Five GSDIa image datasets from the University of Florida College of Medicine and three healthy adult image datasets from Philips Research North America (PRNA) were used for this study under informed consent. All patient datasets were de-identified following PRNA procedures.

3D model construction and simulations were performed in MATLAB (The MathWorks Inc, MA). A custom tracing program was used to extract the tissue types of interest from the subject image datasets (bone, liver, and soft tissue). Once these structures were identified, a unique numeric value was assigned to each tissue type (Table 1) and a 3D abdomen model matrix generated for each subject. All models were re-sampled to a uniform voxel size of 2mm, providing a reasonable balance between accuracy and analysis time. For the initial geometric analysis, the FUS beam was modeled following the “cone of entry” approach used in [3]. A transducer face layer was added to prevent immersion of the transducer matrix into the abdomen model matrix during the simulated transducer scanning and rotation motions.

| Table 1. Numeric values for matrix elements and their combinations |
|-------------------|-----------------|-----------------|
| Transducer Beam (1) | Focal Zone (2) | Transducer Face (18) |
| Soft Tissue (3) | 4 | 5 | 21 |
| Liver (6) | 7 | 8 | 24 |
| Bone (9) | 10 | 11 | 27 |

The scanning algorithm was run on a Dell PC with 4 CPUs (2.8GHz) and 4GB of memory. Briefly, the translated and/or rotated transducer model was combined with the abdomen model via matrix addition. The acoustically accessible liver volume in this analysis was defined as the region of the liver that can be reached by the focal zone of a particular transducer (‘8’), without any part of the beam interfering with
bone (‘10’, ‘11’, and ‘27’), while the transducer face does not interfere with any body structure (‘21’, ‘24’, ‘27’), divided by the total liver volume (‘6’). If this criterion is met, the particular liver voxel is marked as accessible. The scanning algorithm scanned the transducer focal zone through all liver voxels, simulating a USMD treatment. This process was repeated after rotating the transducer beam matrix along the x and y axis by ±30°, ±45°, and ±60° across all abdomen models, while varying the transducer aperture from 30mm to 100mm (in 10mm increments), and focal lengths from 60mm to 140mm (in 10mm increments). Once transducer candidates that could access a desired minimum liver volume based on the geometric analysis were identified, acoustic field simulations were performed for these candidates using finite element analysis software (PZFlex, Weidlinger Associates Inc, CA) to evaluate these transducers’ capabilities to also generate the required acoustic pressures (2.5 MPa [4]) at their focal zone for reliable USMD treatments. The model used for these simulations (Figure 1) consisted of a skin, fat, and liver layer.

![Acoustic field simulation model](image)

**FIGURE 1.** Acoustic field simulation model. Model geometry (left) and resulting peak pressure field (right) is shown for a 60mm aperture/120mm focal length transducer ($I_{\text{transducer surface}} = 10 W/cm^2$).

Twelve elements per wavelength meshing were used for each model, and all boundaries were set to be absorbing. A Dell PC (Dell T5500, Round Rock, TX) with a dual-core 64 bit 2.66GHz Intel processors and 12GB memory was used for all acoustic field simulations. The transducer operating frequency was kept at 1MHz, and the intensity at the surface of the transducer was kept below 10W/cm² [5]. An ultrasound wave consisting of a single pulse was simulated, and the peak acoustic pressure was calculated for the entire field.

**RESULTS**

Figure 2 shows the acoustically accessible liver volume for GSDIa patients and healthy adult subjects, computed as a function of transducer aperture and focal length,
based on the geometric analysis alone. Approximately 70% of the transducers examined were able to access 30% or more of the liver in the GSDIa patients, while 30% of them were able to access 50% or more of the liver in those patients. When the acoustic pressure simulation results were considered as well, 40% of the transducers examined were able to access 30% or more of the liver in the GSDIa patients, while only 5% of them were able to access 50% or more of the liver in these patients. This last group of transducers included the f/2 transducers of 120mm focal length/60mm aperture, and 140mm focal length/70mm aperture, for example.

Not surprisingly, GSDIa patients in general had a larger acoustic accessible liver volume than the healthy adult subjects, due to their increased liver size and protrusion from under the ribcage. Figure 3 highlights this approximate 3-fold difference, showing the accessible liver volume for both GSDIa and healthy subjects as a function of focal length for a transducer with a fixed 100mm aperture.

FIGURE 2. Acoustic accessible liver volume as a function of focal length and aperture for GSDIa patients (left) and healthy adult patients (right), based on geometric criteria alone.

FIGURE 3. Mean accessible liver volume for GSDIa patients and healthy adult subjects as a function of transducer focal length for a fixed 100mm transducer aperture (result of geometric analysis only).
DISCUSSION

USMD has been intensively investigated in recent years, and a large acoustic window definitely benefits therapeutic outcomes in the treatment of various diseases. With a sufficiently large acoustic window, physicians would have much more flexibility in transducer positioning and treatment execution. Just as for HIFU ablative treatments using focused ultrasound, an adequate acoustic window is a requirement for effective gene transfection for GSDIa patients as well. Currently, it is believed that 10% - 30% liver transfection is required to achieve effective synthesis of G6Pase enzyme [6]. The results from this study provided the first quantitative evaluation of accessible liver volume in adolescent GSDIa patients, considering both geometric as well as acoustic delivery constraints. Several transducer geometries were able to meet both of these constraints, showing the feasibility of USMD (using either single element or ultrasound arrays) for pDNA delivery in GSDIa applications.

CONCLUSION

This study provided an efficient method to quantitatively estimate acoustic windows for USMD in GSDIa patients. A geometric approach was chosen to compute the acoustically accessible liver volume for various transducer geometries, and acoustic field simulations were performed to confirm capabilities of transducers to generate pressures of therapeutic levels. The algorithm used in this study can be generalized for acoustic accessibility estimation of other targets as well. The conservative evaluation results presented in this study would be beneficial for physicians during transducer selection and treatment planning, once the desirable liver tissue to be treated is known. Meanwhile, transducer designers could use this data for balancing necessary acoustic energy and transducer requirements during the design process.

REFERENCES