Immune reactions associated with silicone-based ventriculo-peritoneal shunt malfunctions in children

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Abstract

The implantation of ventriculo-peritoneal (VP) shunting systems is the most commonly performed neurological procedure in children with hydrocephalus. Although the overall complication risk is low, the cumulative risk of shunt failure is high and unfortunately results in a high prevalence of revision surgeries. In this study, we explored the concept that some pediatric patients may develop an immune response to either the proteins attached to the silicone implant surface or to the biomaterial itself, and that this reaction may contribute to VP shunt failure in some individuals. The data displays that the sterile shunt malfunction group had a higher rate of protein deposition and increased levels of autoantibodies to the extracted surface proteins as compared to individuals with functioning shunting systems. The precise nature of the shunt-bound proteins that serve as antigens in this experiment have not yet been determined. The data also indicated that some individuals develop antibodies to polymeric substances that cross-react with partially polymerized acrylamide. The detection of significant amounts of shunt-bound protein, antibody responses to these proteins and to polymeric substances suggest that an immunological response to these proteins may play a role in the mechanism behind sterile shunt malfunctions.

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1. Introduction

The implantation of shunting systems is the most commonly performed neurological procedure in children, with approximately 18,000 new devices placed annually [1]. Hydrocephalus is a congenital or acquired condition where an accumulation of cerebral spinal fluid (CSF) within the brain arises, usually due to an imbalance between the generation and absorption of fluid. Currently, the most effective treatment of hydrocephalus is a silicone CSF shunting system, with the most common being the ventricular-peritoneal (VP) shunt. These shunts function to relieve pressure caused by the accumulation of excessive CSF in the brain, and drain the fluid to a non-constrained area of the body, such as the peritoneal cavity. The use of silicone-based VP shunts for CSF shunting has dramatically improved the outlook for individuals with hydrocephalus. Unfortunately, the long-term need for revision of silicone shunts results in a high accumulation of shunt related complications. The rate of shunt failure in the first year after implantation is around 30%, and it has been estimated that the likelihood of a shunt to still be functional in 10 years is only 15% [2]. There are more than 125,000 CSF shunt procedures performed in the United States alone, accounting for national health care spending of almost $100 million each year [1]. While VP shunts are a necessity for treating these children, many complications occur repeatedly after implantation, with sterile malfunctions of silicone shunts remaining a major complication. One report determined that proximal occlusions of the VP shunts accounted for 47% of
revisions [3]. Another report concluded that 56% of primary shunt failures were caused by shunt blockage [4]. Several other investigations suggest that obstructed shunt failures may be attributed to either the ingrowth of tissue into the catheter of the shunt [5], a non-specific cellular and humoral immune reaction [6,7], or shunt rejection from a hypersensitivity reaction [8,9]. While immunological reactivity to silicone remains a controversial issue unresolved by the silicone breast implantation investigation and litigations, the possibility of silicone behaving as an immunological adjuvant or reactive biomaterial surface remains a possibility with many reports finding immune reactions [10–17]. Clinical data also elevate confusion by demonstrating antibodies to differing forms of silicone. Some state that these antibodies are not linked to adverse pathological conditions at the time of study, however, cannot refute the chance of future problems [18–24]. Despite concerns raised, we find it appropriate to investigate the response, if any, to the silicone shunt system. In this study, we explored the concept that some pediatric patients may develop an immune response to either the proteins attached to the implant surface or to the biomaterial itself, and that this reaction may contribute to VP shunt failure in some individuals. Both direct reactivity to the polymer and reactions to silicone bound proteins are investigated.

2. Patients and methods

2.1. Patients

Silicone-based VP shunts were recovered from 39 children (female n = 18, male n = 21) requiring surgical shunt revision. Patients ranged from 2 to 20 years of age, with the average age as 10.5 years. Three groups were identified based on the diagnosis for surgical revision: sterile malfunctions (n = 24), malfunctions due to infectious causes (n = 8), and non-malfunctioning elective shunt lengthening controls (n = 7). All VP shunt systems were comprised of medical grade silastic tubing and were produced by the same manufacturer (Medtronic PS Medical; Goleta, CA). A portion of the distal shunt tubing and serum were collected from each patient during surgery. The shunts were placed in sterile phosphate saline buffer (PBS), while the serum was stored at −80°C. All procedures were approved by the Human Investigation Committee of Wayne State University.

2.2. Isolation and preparation of proteins

Once the distal portions of the shunts were collected, they were washed three times with sterile PBS at 4°C for 3 days to remove the loose tissue. A glycine solution was used to remove the covalently attached proteins, followed by a stringent guanidine wash to remove the proteins with a higher binding affinity to the silicone. Proteins from the silicone shunts were extracted by immersion in 15 ml of glycine/HCl buffer (pH 2.8) and vortexed for 10 min. The protein solution was removed and was adjusted to pH 7.5 by addition of 3 M Tris buffer. Proteins were next extracted from the samples by guanidine. Again the shunts were vortexed and the protein solution neutralized. All protein solutions were placed into Centriprep concentrators (Amicon, Beverly, MA) to concentrate proteins above a molecular weight of 10 kD. Centripreps were centrifuged at 2000 g for 1 h, and the amount of protein per length of shunt was determined using the BCA protein assay (Pierce; Rockford, IL) in accordance with the manufacturer’s instructions. A protein standard of a known concentration, bovine sera albumin, was used to accurately calculate the protein concentration.

2.3. Immunoblotting assays

The dot blot technique was used to identify if the patient serum contained any antibodies to the autologous shunt proteins. Briefly, 10 mg of each shunt protein extract was transferred onto a nitrocellulose membrane (Amersham, Arlington Heights, IL) in duplicates using a vacuum dot blot apparatus (Bio-Rad Laboratories; Hercules, CA). Blots were then incubated in blocking solution of 5% milk/PBS for 1 h, and then washed three times with PBS/0.05% Tween for a total of 15 min. Serum samples from the shunt revision patients were diluted 1:500 in 5% milk/PBS and the extracted protein blots were incubated with autologous sera overnight at 4°C. After incubation, the blots were washed and then incubated for 2 h at room temperature with a solution of goat anti-human Ig conjugated with alkaline phosphatase diluted 1:2000 in 5% milk/PBS. The blots were again washed, and then developed by the addition of dissolved BCIP tablets (Sigma, St Louis, MO) in distilled water. As a control, anti-human Ig conjugated with Alkaline Phosphatase was also tested against the shunt protein using the same technique to determine the levels of immunoglobulin extracted from the surface of the shunts. Autoantibodies were calculated by subtracting the control data from the autologous sera data. Digital images of the blots were acquired using the Alpha Innotech camera system, and the intensity of color was measured using the Image Pro Plus analysis software package (AlphaInnotech, San Leandro, CA). As a control to negate the possibility of cross reactivity, immunoblots of human serum albumin (HSA) were also incubated with patient sera, following a similar protocol as above.
2.4. Anti-polymer antibody (APA) assay

Anti-polymer antibody (APA) binding was assayed by Autoimmune Technologies (New Orleans, LA) using two formats of the assay. The APA assay detects antibodies to polymeric substances that apparently cross-react with partially polymerized acrylamide [25]. Shunt patient sera were evaluated using the ELISA format of the assay. Briefly, aliquots of partially polymerized acrylamide were diluted in distilled water and applied to 96-well ELISA plates and allowed to dry. The wells were blocked with 5% milk/TBS for 45 min prior to the addition of diluted patient sera, in triplicate, followed by a further incubation of 90 min. All incubations and washes were carried out at room temperature. The wells were washed and a secondary antibody, biotin conjugated goat anti-human IgG (KPL, Gaithersburg, MD) was added at a dilution of 1/1000. After an incubation of 60 min, the wells were washed and blocking buffer containing avidin conjugated horse radish peroxidase (Jackson Immunoresearch, West Grove, PA) diluted 1/500 was added. Following another 60 min incubation, the wells were washed again, and a detection buffer was added. The reaction was allowed to proceed 15 min, and then read using a microplate reader at optical density (OD) 405. A positive calibrator control, a strong positive, weak positive, negative and blank were run on each plate in triplicate. After subtracting the average blank OD, OD values for the patient sera were averaged and then normalized to the background-subtracted mean OD of the calibrator.

The APA strip assay was used to characterize the binding of APA to antigen as previously described [8]. Briefly, aliquots of partially polymerized polyacrylamide were sequentially diluted 10, 100, and 1000 fold in dH2O for 1/1000. After an incubation of 60 min, the wells were washed and blocking buffer containing avidin conjugated horseradish peroxidase (Jackson Immunoresearch, West Grove, PA) diluted 1/500 was added. Following another 60 min incubation, the wells were washed again, and a detection buffer was added. The reaction was allowed to proceed 15 min, and then read using a microplate reader at optical density (OD) 405. A positive calibrator control, a strong positive, weak positive, negative and blank were run on each plate in triplicate. After subtracting the average blank OD, OD values for the patient sera were averaged and then normalized to the background-subtracted mean OD of the calibrator.

3. Results

3.1. Protein analysis

The mean level of protein bound to silicone shunts was 18.13 ± 1.84 μg/cm. Analysis revealed no significant difference among the patient groups under investigation, although the highest level of protein binding to silicone was observed in patients within sterile shunt malfunctions (19.9 ± 2.68 μg/mg) and the lowest level was seen in patients with infection associated malfunctions (12.1 ± 1.81 μg/mg). These data suggest that binding of protein to silicone is constant irrespective of the underlying reason for shunt revision. The time of shunt implantation for the control group (95.14 ± 12.03 months) was significantly different (p < 0.001) when compared to both the sterile shunt malfunction (6.06 ± 2.04 months) and infection groups (5.65 ± 1.24 months). When the protein data was reanalyzed to examine whether the time of implantation influenced the protein deposition, statistical significance was achieved. Both the malfunction (136.1 ± 27.7) μg/mg/year) and the infection (175.7 ± 69.8 μg/mg/year) groups displayed a higher rate of protein deposition as compared to the elective lengthening group (3.14 ± 0.78 μg/mg/year) (p < 0.01) (Fig. 1).
3.2. Immunoblot analysis

The dot blot assay revealed that the majority of VP shunt patients (24/39) exhibited a positive antibody reaction to silicone bound proteins. One VP patient serum resulted in a low positive reaction when using HSA as the control immunoblot antigen. Further, analysis of the extracted shunt protein indicated that immunoglobulin comprised a large component of the extracted shunt protein in nine of the 39 patients. Interestingly, of these nine patients with antibodies that bound directly to the silicone, eight were positive by the APA test. The data in Table 1 classifies the positive patients by study group. These positive antibody reactions are very different from the positive immunoglobulin findings in the protein extracts. These results suggest that immunoglobulin was attached directly to the silicone shunts, and thus became extracted with the rest of the proteins attached to the implant. However, a positive dot blot assay reading indicates that the patient has developed antibodies to the autologous proteins attached to the implant. In these 9 patients, positive reactions due to the directly bound antibody (now present in the antigen extract) complicated the interpretation of autoantibody levels in these sera, since these patients displayed a higher binding to the anti-human Ig control as compared to reaction with their autologous sera. When the data was corrected for this complication, patients from the sterile shunt malfunction group were seen to have the highest autoantibody response (OD = 7.87(±1.81)), which was significantly higher (p > 0.03) than the antibody binding that occurred in the elective lengthening (control) group (OD = −1.5 (±5.61)). This negative value corresponds to higher OD value of the control blot as compared to the blot incubated with patient sera, thus the average of the control group demonstrated the low levels of autoantibodies present within their sera (Fig. 2). Antibody binding in patients with infection-associated malfunctions was elevated over the levels seen in the control group (OD = 6.8(±2.57)). However, this increase did not achieve statistical significance. Antibody reactions to bound proteins were classified according to the level of the optical density units observed in each individual reaction as either negative (OD = 0), high (OD > 16), moderate (OD = 8−16) or low (OD = 0.1−8). This classification defined 15 patients as negative, 6 patients with low reactivity, 12 patients with moderate reactivity and 6 patients with high reactivity.

3.3. Anti-polymer assay (APA) ELISA

The APA ELISA assay indicated that the majority of VP shunt patients (30/39) exhibited a positive serum antibody reaction to partially polymerized polyacrylamide. In this test, the highest APA response was observed in patients with infection-associated malfunctions (2.47 (±0.55)), followed by the sterile shunt malfunction group (1.25 (±0.32)) and the control group (0.53 (±0.33)) (Fig. 2). Patients with infected shunts developed significantly elevated APA binding when compared with the level of antibody binding observed in the elective lengthening (control) group (p<0.012). Interestingly, the level of APA binding in patients with infection-associated malfunctions was also significantly elevated over the sterile shunt malfunction group (p<0.041). Using the individual antibody binding OD units, antibody reactions to partially polymerized

![Graph](Image)

**Fig. 1.** Graph depicts the amount of proteins extracted (µg/cm) from the surface of the silicone VP shunts (black) and the amount of protein when implantation time is incorporated into the data (gray). Diagnosis group classifies patients. The sterile shunt malfunction group has the highest levels of proteins extracted from the surface and the data is exacerbated when the time factor is addressed. Thus, rate of protein deposition is significantly higher with the experimental groups as compared to the elective lengthening (control) group (p<0.01).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Table displays the categorization of reactive patients by diagnosis group</th>
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<tbody>
<tr>
<td>Sterile malfunction</td>
<td>Infection</td>
</tr>
<tr>
<td>Positive immunoblot patients (n = 24)</td>
<td>14</td>
</tr>
<tr>
<td>Number of patients presenting with extracted immunoglobulin (n = 9)</td>
<td>4</td>
</tr>
</tbody>
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The first row displays the individuals who had a positive autoantibody response to their protein extracts. The second row displays the number of individuals whose protein extract contains immunoglobulin. Data was determined using the immunoblotting procedure as described in the Section 2.
polyacrylamide were classified as negative (OD < 0.1), low (OD = 0.1 – 1.0), moderate (OD = 1.0 – 3.0), and high (OD > 3.0). This classification of the patient responses generated the following stratification of the findings: negative responses (9 patients), low responses (14 patients), moderate responses (8 patients), and high reactivity (8 patients). There was no correlation found between levels of antibodies to silicone bound proteins and the presence or levels of antibodies to APA. There was also no correlation between the levels of APA and protein extract immunoglobulin.

3.4. Characterization of APA IgG

To demonstrate that the binding of antibody to partially polymerized polyacrylamide was specific, APA strip assays were performed in which 10-fold serial dilutions of the antigen were included as a competitor. The data in Fig. 3 demonstrates that excess partially polymerized polyacrylamide effectively competed for antibody binding to the assay strips with no detectable levels of non-specific binding (<5.0% at 1:100 dilution of competitor) at the highest concentration of competitor used. Partially polymerized polyacrylamide had no effect on the binding of anti-HIV antibodies to HIV antigens on the HIV western blot at the concentrations used here demonstrating that the competition of partially polymerized polyacrylamide for the binding of APA was specific (data not shown).

To determine if the binding of APA to partially polymerized polyacrylamide was independent of total IgG concentration and the presence of non-IgG serum proteins or factors, total IgG was purified from both APA positive and negative patient sera samples using a protein A column. Aliquots containing up to 21.4 mg of purified IgG from each of the samples were then analyzed using the APA strip assay. To exclude the possibility that any serum component or non-IgG-related protein was involved in binding immunoglobulin to the strips, the blocking buffer used during initial incubation of the strips with the purified IgG fractions was changed to a formulation that did not contain any serum or protein (PBS plus 0.1% Tween 20). (Goat serum is routinely used as a component of the blocking buffer in the APA assay.) Purified IgG from a seropositive patient retained the ability to bind to partially polymerized polyacrylamide, while purified IgG from an APA seronegative patient failed to bind antigen at any concentration of IgG tested (Fig. 4). These results demonstrate that the binding of APA-specific IgG to partially polymerized polyacrylamide was independent of total IgG concentration and independent of non-IgG related serum components, including albumin.
3.5. Association of immune reactions with the clinical aspects of shunt malfunction

The presence of atopic disease in the patients failed to correlate with either the amount of extracted shunt protein, or the antibody responses to extracted protein or APA levels. The presence of common or seasonal allergies did not influence the level of protein binding or the development of antibodies, and there was no significant difference between the groups. Investigation of implantation time and number of revision surgeries revealed statistically significant differences between groups. As might be expected, the implantation time was significantly less for the shunt malfunction groups as compared to elective control group ($p<0.001$). The number of revision surgeries per each patient was also less for the control group as compared to sterile shunt malfunctions and infections as shown in Table 2.

4. Discussion

Many VP shunt patients require multiple revisions due to repeated implant malfunction and local complications [26]. In our study for instance, one patient had 97 revisions since 1992 while another, a 4 year old, had 35 revisions. One group examined primary shunt failures and found that 56% of these were caused by shunt blockage [27]. The choroid plexus, brain debris, fibrin, and clotted blood were found to be the most common elements of blockage. Variations in the proximal tip and tubing size have been tested, as well as the addition of flanges, however none have helped to solve the problem [28]. In addition to mechanical failure and failure due to infection, several studies have found that sterile shunt malfunction, in which failure of the shunt cannot be attributed to infection or mechanical failure, accounts for up to 30% of all shunt failures [29,30]. Frequently, shunts malfunction for unknown reasons. Some researchers have alluded to the possibility of obstruction due to an immune response to the biomaterial. Hypersensitivity reactions to silicone have been previously reported in other clinical applications [7–9]. This immune reaction may progress slowly and appears to correlate to eosinophilic reactions to a sterile shunt system. One group reported on a child with hydrocephalus who had numerous VP shunt malfunctions at a young age [7]. Although the cause is still unclear, that child developed latex allergy and CSF eosinophilia. A systemic glucocorticosteroid treatment, which inhibits the transcription of specific cytokines related to eosinophil priming and chemotaxis, effectively reduced CSF eosinophilia and decreased the shunt malfunction rate. A further study examined the internal surface of blocked shunt systems removed because of malfunction [8]. Using scanning electron microscopy, they revealed a variety of cells adherent to the silicone-tubing wall. Several shunts contained neutrophils and giant cells with multiple foot processes adherent to the surface. These authors hypothesized that their findings represented a delayed hypersensitivity reaction to silicone. These results suggest that some individuals may elicit an immune response to this biomaterial. Goldblum et al. demonstrated that sera from some VP shunt-implanted individuals contained antibodies to silicone [31].

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**Table 2**

Table displays the categorization of patient by diagnosis group

<table>
<thead>
<tr>
<th></th>
<th>Sterile malfunction</th>
<th>Infection</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average implantation</td>
<td>6.05</td>
<td>2.10</td>
<td>95.14</td>
</tr>
<tr>
<td>time (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of revision</td>
<td>16.61</td>
<td>34.71</td>
<td>4.71</td>
</tr>
<tr>
<td>surgeries per patient</td>
<td></td>
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</table>

The first row displays the average time in months of shunt implantation per patient. The second row displays the mean number of revision surgeries each patient endured up to the time of shunt collection.
This group concluded that the immune system could recognize and mount a specific response against silicone. Again, this study also hypothesized that an immune response could lead to shunt malfunction in certain children that are continually exposed to silicone.

Our data suggests that there are significant differences between the non-malfunctioning elective shunt lengthening controls and the sterile shunt malfunction patients. Although no statistically significant difference was found when examining the amount of extracted protein, taking into account the length of time implanted certainly increased the amount of protein extracted off the sterile shunt malfunction, while lessening the amount extracted from the control group. Thus, the rate of protein deposition is significantly increased with the experimental groups as compared to the non-malfunctioning elective shunt-lengthening group. Our data also indicates that a high percentage of patients with silicone shunt malfunctions requiring revision surgery develop an antibody response to proteins extracted from the surface of the shunt. In addition, we demonstrated that APA, which has been proposed to be a silicone-associated response [32], are present in the majority of shunt patients studied. These results suggest that there may be an association between antibodies to either the proteins attached to the shunt or the polymer and sterile shunt malfunction. The occurrence and level of these antibodies were not correlated, suggesting different mechanisms in the development and specificity of the response. The precise nature of the silicone bound proteins that serve as antigens in this experiment have not been determined, since we were limited by the very low levels of protein recovered, which did not permit immunological or biochemical analysis. Additional studies are also required to determine if APA titers can be used to predict if a patient will be or is at risk of developing sterile malfunction of their shunt. The clinical significance of the antibody responses also remains to be determined. Although CSF shunts have generated a dramatic improvement in clinical symptoms, the long-term results still show a high incidence of shunt related complications.

5. Conclusion

The detection of significant amounts of shunt-bound protein and antibody responses to these proteins and to polymeric substances suggests that an immunological response to these proteins may play a role in shunt malfunctions. The lack of correlation between the two antibody responses suggests different mechanisms in the development and the specificity of the immune response. Further research is required to characterize the precise nature of the silicone bound proteins. Understanding this biochemical and immunologically relationship will allow for biomaterial surface modification and possible host acceptance.

References


