Leptomeningeal inflammation in infant and foetal deaths without trauma

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Received: July 01, 2014
Published online: September 11, 2014

The words ‘without trauma’ are the most important in the title of this article. It has been a long-held belief that the presence of inflammatory cells in the leptomeninges or dura is a reliable marker for a traumatic process. However, there is no evidence in the literature to support this claim, thereby calling into question the reliability of inflammation as a forensic marker of trauma.

Most studies of the mediators of inflammation in brain injury examine the cerebrospinal fluid and brain proper, utilizing cohorts of individuals with traumatic brain injury [1, 2, 3]. The assumption that the presence of inflammatory cells in the leptomeninges in individuals with traumatic brain injury is a direct consequence of that traumatic process is an extrapolation of these studies. This extrapolation is dangerous as there is a dearth of information about the normal inflammatory cellular constituents of the leptomeninges outside the setting of trauma [4].

Recognizing a paucity of information regarding the leptomeninges and inflammation, we sought to define a baseline level of inflammation that may be present in a population of foetuses and infants in the absence of trauma (other than birth-related trauma). Our cohort was derived from foetuses and infants who were autopsied at a large academic hospital (Lucille Packard Children's Hospital (part of Stanford University Medical Center), California, USA). We aimed to quantify the presence of inflammatory cells as well as iron-containing cells as a means of establishing a reference point for use in evaluating forensic cases where trauma may or may not be a consideration.

We know from previous studies that the dura in early gestation and the first few postnatal months is dynamic [5]. Recognizing this dynamic state, we subdivided our study population into two groups - those who survived greater than 33 days up to a year of age (with a single exception; group 1) and foetal deaths in the late third trimester up to 33 days of post-natal life (with two exceptions; group 2). We sought to describe the inflammatory cellular constituents in the combined cohort and compared the results between these two subgroups. We also attempted to
correlate our findings with other features (e.g. mode of delivery) and diagnoses from the general autopsy and neuropathologic examination.

Our study included 33 foetal and infant autopsy cases from the Lucille Packard Children’s Hospital. The general autopsies were performed by the residents in pathology and attending pathologists. The neuropathologic examinations were performed by the same neuropathologist in all cases. To ensure a wide sampling of leptomeninges for each case, at least two or three brain sections (cerebral cortex, cerebellum, brain stem) containing at least 5 mm of leptomeninges each were examined. To assist in detection of the inflammatory cells, immunohistochemical staining was performed utilizing antibodies to the following antigens: CD45, CD68 and CD163. CD45, also known as leucocyte common antigen, is present on neutrophils, eosinophils, basophils, lymphocytes, and monocytes. CD68 is present on macrophages/monocytes, and CD163 is present on cells of the monocytic lineage and microglia (and has substantial overlap with CD68).

The method employed in quantifying the inflammatory cells was quite straightforward; the length of the leptomeninges was first measured and the appropriately staining cells within this length were discretely counted. Intravascular cells which displayed appropriate staining were not included in our counts as we were only interested in the inflammatory cells present intrinsically within the leptomeninges. Examples of appropriately stained and localized cells are demonstrated in Figures 1, 2 and 3. The density of inflammatory cells (in each immunostained section) or iron containing cells per length of leptomeninges (measured in mm) was then calculated in each region of the brain. The mean number and standard error of the mean for each stain in the two groups was then calculated.

We evaluated a total of 33 cases - 16 of which were in Group 1, and 17 in Group 2. There were 16 males and 17 females. Thirteen cases were delivered vaginally, and 19 were delivered through Caesarean-section (the mode of delivery of one case was unknown).

The general autopsy and neuropathological diagnoses of both groups varied and overlapped. In Group 1 there were four cases of congenital heart disease and four additional cases of congenital non-cardiac malformations while in Group 2 there were 8 cases of congenital heart disease and 3 additional cases of congenital non-cardiac malformations. Some of the malformations in each group were part and parcel of chromosomal abnormalities as well; small numbers of additional cases involving chromosomal abnormalities were present in each group. Two cases of sepsis or significant infections were present in two cases in each group. Some form of hypoxic/ischaemic event (e.g. pontosubicular neuronal necrosis, infarctions, periventricular leukomalacia, etc.) was found in 11 cases in Group 1 and 10 cases in Group 2. Two cases in Group 1 and seven cases in Group 2 had some form of haemorrhage involving the epidural (group 1 only), subdural or subarachnoid compartments.

In total, we examined 39 slides from the cerebral cortex,
37 from the brain stem, and 30 from the cerebellum - the leptomeningeal length of these ranged between 5-41 mm, 10-83 mm, and 10-47 mm, respectively. These figures reflect a wide sampling range from different brain regions across all samples.

In Group 1, the mean number of immunoreactive cells per length of leptomeninges across the three brain regions were as follows: for CD45 there were 21.49 cells/mm in the cortex, 10.96 cells/mm in the brain stem and 9.63 cells/mm in the cerebellum; for CD68 there were 25.74 cells/mm in the cortex, 13.13 cells/mm in the brain stem and 9.26 cells/mm in the cerebellum; and for CD163 there were 31.89 cells/mm in the cortex, 14.98 cells/mm in the brain stem and 15.1 cells/mm in the cerebellum.

In Group 2, the mean number of immunoreactive cells per length of leptomeninges across the three brain regions were as follows: for CD45 there were 13.13 cells/mm in the cortex, 12.63 cells/mm in the brain stem and 11.76 cells/mm in the cerebellum; for CD68 there were 16.64 cells/mm in the cortex, 18.81 cells/mm in the brain stem and 15.87 cells/mm in the cerebellum; and for CD163 there were 22.61 cells/mm in the cortex, 23.37 cells/mm in the brain stem and 19.53 cells/mm in the cerebellum.

We performed statistical tests to determine if there were indeed any significant differences between the two subgroups in terms of the mean numbers of iron containing and immunoreactive cells in the brain regions. We found no statistically significant difference in the mean numbers of iron containing or immunoreactive cells per length of leptomeninges between the groups.

Notably, three cases with no reported neuropathology (one case in Group 1 and two in Group 2) demonstrated inflammatory cells in all brain regions studied. This is a novel finding demonstrating that inflammatory cells may be found in the leptomeninges not only in cases without trauma, but also in those without any neuropathological findings.

With regards to the deposition of iron, we found detectable iron in the leptomeninges of cases born via both vaginal delivery and Caesarean section. We detected iron in the leptomeninges of sixteen of 19 Caesarean section deliveries and in eight of the 13 vaginal deliveries. Thus, the presence of iron does not absolutely suggest postnatal traumatic haemorrhage but may be found in completely naturally occurring processes and occurs irrespective of the mode of delivery.

Now that we have established that inflammatory cells can be present normally in the leptomeninges and in the setting of varied natural disease processes, we are faced with the question of the function of the inflammatory cells under normal conditions.

There is evidence for various means allowing the brain to sense inflammatory signals in areas devoid of a blood-brain barrier. It could be postulated that these leptomeningeal inflammatory cells (particularly those of macrophage and monocyte lineage) become activated in response to distant damage through the systemic circulation [7].

From our study, it is apparent that the presence of iron in the leptomeninges does not always equate to postnatal traumatic haemorrhage. The iron found within the leptomeninges could be a result of the development of bridging veins early in gestation, wherein plexiform veins in the dura resorb and anastomose to eventually become definitive bridging veins in the first few months of postnatal life[5, 8].

We conclude and have proven that foetal and infant
leptomeninges contain significant numbers of inflammatory cells in a variety of natural and non-traumatic disease processes. Furthermore, iron deposition may be found in the leptomeninges in atraumatic conditions and could possibly be a consequence of normal development. The presence of inflammatory cells alone are therefore not a reliable marker of remote or acute trauma in the forensic setting. The presence of inflammation in the leptomeninges should prompt the pathologist to scrutinize the child's developmental and medical history for a disease process or condition, such as those specifically described above, which could account for the inflammatory infiltrate[6].

To place our findings in a more forensically relevant setting, we intend to apply a similar methodology as described above to cases arising in medical examiner/coroner populations. We plan to initially compare our findings in hospital-based cases with infants dying in a forensic setting including natural disease processes or without a definable, specific cause of death (e.g. sudden infant death syndrome/sudden unexplained death in infancy). This forensic population will help determine a more relevant forensic baseline upon which cases of inflicted head injury can be compared.

References