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RESEARCH ARTICLE

ORAL MICROBIOTA OF NEW BORN INFANT OF CAESAREAN & VAGINAL DELIVERIES: A COMPARATIVE MICROBIOLOGICAL STUDY.

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Abstract

Aim:- To investigate the correlation between the bacterial flora of vagina of the mother and the oral cavity of the newborn and to determine the difference in the oral microflora of a newborn with normal and cesarean delivery immediately after birth and after feeding.
Methodology:- The study was conducted on 50 expectant mothers. The samples were collected from the 2 Registered Private Maternity Hospitals in Mathura City. The subjects were informed and explained about the procedure and a written consent was obtained. The samples were collected from 25 newborns by normal delivery and 25 by cesarean delivery. Three swabs were taken from the subjects i.e., one from maternal birth canal and 2 from the oral cavity of newborn immediately after birth and after first feeding. Samples were sent to the laboratory under sterile condition, for the microbiological assessment.
Results: In this study it was observed that the oral cavity of newborns was found to be sterile in 40% of normal delivery and 76% through caesarean delivery.
Conclusion:- The mode of delivery has an influence on the salivary microbial profile in infants. Streptococcus salivarius was established into the oral cavity only after the first feeding.

Background:-

Myriads of micro organisms abound in the world. These single cell creatures represent the major diversity of life on our planet. Micro organisms not only constitute the communal but also the normal microflora of the human body. The human body, consisting of about 100 trillion cells, carries about ten times as many microorganisms in the intestines.[1] The healthy human fetus is thought to develop in a bacteria free environment. It was previously hypothesized that the oral cavity of the new born is sterile at the time of birth and the colonization starts after the neonate is exposed to the environment.

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But studies have shown that upon delivery the neonate get exposed to a wide variety of microbes which are provided by the mother during and after passage through the birth canal.[2,3,4,5] These micro organisms participate in the initial building of the oral ecosystem and also produce changes in the long term colonization of oral microbiota as well as subsequent alteration in the immune development of the new born.[6]

Hence the aim of this study was to investigate whether there is a correlation between the bacterial flora of vagina or amniotic fluid of the mother and the oral cavity of the newborn and also to determine the difference in the oral microflora of a newborn with normal delivery and cesarean delivery immediately after birth and after feeding.

Methodology:-

Before the commencement of this study the clearance was taken from the ethical committee board of K.D. Dental College & Hospital, Mathura. The samples were collected from the 2 Registered Private Maternity Hospitals. The participants of the study were explained about the procedure and informed written consent was obtained. This study was conducted on 50 newborns and their mothers. Information on the past medical and pregnancy history was taken from the mother. Mothers with the history of recent antibiotic usage, viral and systemic diseases were excluded from this study.

Prior to the delivery, swabs were taken from the mother's birth canal using a sterile cotton swab. Immediately following the birth & 2 hours after the first feeding, swabs were taken from the oral cavity (tongue & buccal musosa) of the newborn. All the samples were collected aseptically in duplicate with sterile swabs and transported to laboratory at the earliest.

The swabs were inoculated in tryptose soya broth(TSB) and incubated at 37°C for 24 hours at two different conditions. One set was incubated in ordinary incubator while second set was incubated under 5% CO_2 tension in desiccators for the isolation of microaerophilic as well as facultative anaerobic bacteria pathogens.

After incubation the broth showing the turbidity were transferred on Tryptose soya agar medium culture plates with the help of sterile bacteriological loop and further incubated at two different incubation conditions viz., aerobic and 5% CO_2. The glass slides with the smear were stained with Gram's stain and microscopically observed for organism morphology. The specimens inoculated in the Tryptose soya agar were then placed in the incubator for 18-24 hours and then inoculated into the blood agar and McConkey media, using a sterile inoculating loop. The media were incubated for 18-24 hours. After 24 hours, the culture media were observed for growth. If no growth was evident, it was further incubated. Colonies from the growth were observed, to study colony morphology. The colonies were further processed for sub-culture. A further biochemical reaction was performed to confirm the identity of the organisms. McNemar & Pearson Chi Square test were utilized to compare the data obtained. Thus, a p-value < 0.05 was considered statistically significant to account for multiple comparisons.

Results:-

The micro organisms isolated from the birth canal of the mother included S. epidermidis, S. aureus, Lactobacillus, Kleibsella, Neisseria, P. aeroginosa, Candida albicans, Gardenella sp., E. coli and Micrococcus.

Table 1 shows the highly significant difference in the levels of Lactobacillus and Candida albicans from the mother to child in normal delivery. When comparing the sterile conditions of oral cavity immediately and 2 hours after feeding the difference was found to be highly significant. (*Table 2*)

On comparison of vaginal microflora of mothers with the microflora of newborn of C- section, a significant difference was observed in the levels of S. epidermidis and Lactobacillus. (*Table 3*)

Counts of micro organisms were also found to vary in subsequent follow up and the micro organisms which were not present at the time of birth were observed to colonize the oral cavity after the feeding. (*Table 2, 4*)

The microbial load was found to be lower in cases of newborn immediately after the delivery in the oral cavity of newborns delivered through caesarean delivery. (*Table 5*)

Table 6 shows no statistically significant difference when intergroup comparison was done 2 hours after the feeding.

Discussion:-

During birth and rapidly thereafter, bacteria from the mother and the surrounding environment colonize the infant's oral cavity. Hence it is seen that the oral cavity is not sterile at the time of the birth which is also indicated by the various studies.[2,7,8]

In this study it was observed that the oral cavity of newborns was found to be sterile in 40% of normal delivery and 76% through caesarean delivery. Witkowski (1935), Panesar J. (1997), Hegde S (1998) reported that the oral cavity of the infants were sterile in 2%, 12% and 6% respectively through normal delivery.

Reddy S (2010)[9] in their study observed that the oral cavity of infants through normal delivery was sterile in 20% whereas through caesarean delivery it was 44%. The difference in the sterile condition of the oral cavity of the infants could be because of all the aseptic precautions taken while conducting this study.

Despite of all the asepsis done, none of the birth canal was found to be sterile. Majority of the micro organisms present at the time of delivery constituted the normal vaginal microflora. In this study, the microorganisms found in mother's birth canal were Staphylococcus *epidermidis*, Staphylococcus *aureus*, Lactobacillus, Klebsiella sp., Neisseria sp., Pseudomonas *aeroginosa*, Candida *albicans*, Gardnerella sp., E. coli & Micrococcus, among which S. epidermidis and S. aureus are the normal habitat of the human skin. It was also seen that these two micro organisms does not co- exist at a particular time in the birth canal and the oral cavity.

Similar micro organisms were also found in the oral cavity of the newborn delivered via normal delivery. This suggests that the mother's birth canal could have been the source for these microorganisms for the child. A similar finding was reported by Tones-Alipi (1990)[10] & Dominguez-Bello (2010)[11]. The degree of contamination of the newborn is significantly correlated with the microorganisms found in the mother's birth canal. Mandar R. (1996)[12] also observed similar findings.

On the other hand, infants delivered via Caesarean delivery harbored microbial communities like Staphylococcus epidermidis, Candida that were most likely to be transferred from the skin of parents, health providers and medical equipments.

The other samples which were taken 2 hours after the feeding, from the oral cavity of the newborns showed a major difference in the microbial colonies of Staphylococcus epidermidis. The bacterium which was not present at the time of birth and was developed after the first feeding was Streptococcus salivarius which is also the initial colonizer of the human oral cavity. Similar results were found by Hegde S (1998) in their study.

Conclusion:-

The following conclusions could be drawn from this study:-

Oral cavity was found to be sterile in 40% and 76% in the newborn through Normal and Caesarean delivery respectively. The mode of delivery has an influence on the salivary microbial profile in infants. Regardless of the type of delivery, the mothers' birth canal was contaminated.

The oral cavity of the newborn may get contaminated with the microorganisms from any of three sources - by contamination from the birth canal or hospital infection or contaminated instruments. Streptococcus salivarius was established into the oral cavity only after the first feeding.

Table I:- Comparison of the levels of micro organisms in the mothers and newborns at the time of delivery (Normal)

	Mother (25)	**Immediately after birth (25)**	**p-value (Mc Nemar Test)**
Sterile	-	10	NA
Staphylococcus epidermidis	20	15	0.062
Staphylococcus aureus	5	0	0.063
Streptococcus salivarius	-	-	NA
Lactobacillus	23	6	**<0.001**
Klebsiella sp.	11	7	0.125
Neisseria sp.	4	9	0.062

Pseudomonas aeroginosa	9	2	0.016
Candida albicans	15	4	**0.001**
Gardnerella sp.	10	6	0.125
E. coli	11	4	0.016
Micrococcus	3	1	0.500

Table II:- Intra group comparison of microbial load in oral cavity of newborn through normal delivery

Bacteria	Immediately after birth	2 Hours after feeding	Immediate v/s After 2 hrs p-value
Sterile	10	2	**0.004**
Staphylococcus epidermidis	15	21	0.016
Staphylococcus aureus	-	-	NA
Streptococcus salivarius	-	6	NA
Lactobacillus	6	9	0.125
Klebsiella sp.	7	3	0.125
Neisseria sp.	9	4	0.062
Pseudomonas aeroginosa	2	-	NA
Candida albicans	4	1	0.250
Gardnerella sp.	6	-	NA
E. coli	4	1	0.250
Micrococcus	1	-	NA

Table III:- Comparison of the levels of micro organisms in the mothers and newborns at the time of delivery (Caesarean)

	Mother (25)	Immediately after birth(25)	p-value (Mc Nemar Test)
Sterile	0	19	NA
Staphylococcus epidermidis	22	6	**<0.001**
Staphylococcus aureus	3	0	0.250
Streptococcus salivarius	-	-	NA
Lactobacillus	16	0	**<0.001**
Klebsiella sp.	-	-	NA
Neisseria sp.	6	0	0.031
Pseudomonas aeroginosa	-	-	NA
Candida albicans	9	2	0.016
Gardnerella sp.	2	0	0.500
E. coli	2	0	0.500
Micrococcus	2	0	0.500

Table IV:- Intra group comparison of microbial load in oral cavity of newborn through Caesarean delivery

Bacteria	Immediately after birth(25)	2 Hours after feeding(25)	Immediate v/s After 2 hrs
Sterile	19	3	<0.001
Staphylococcus epidermidis	6	19	<0.001
Staphylococcus aureus	-	-	NA
Streptococcus salivarius	-	7	NA
Lactobacillus	-	4	NA
Klebsiella sp.	-	-	NA
Neisseria sp.	-	-	NA
Pseudomonas aeroginosa	-	-	NA
Candida albicans	2	2	1.000
Gardnerella sp.	-	-	NA
E. coli	-	-	NA
Micrococcus	-	-	NA

Table V:- Intergroup comparison of the levels of micro organisms in the oral cavity of newborn immediately after delivery

Bacteria	Normal delivery (25)	Caesarean delivery (25)	P-value (pearson's chi-square test)
Sterile	10	19	0.021
Staphylococcus epidermidis	15	6	0.021
Staphylococcus aureus	-	-	NA
Streptococcus salivarius	-	-	NA
Lactobacillus	6	0	0.022
Klebsiella sp.	7	0	0.010
Neisseria sp.	9	0	**0.002**
Pseudomonas aeroginosa	2	0	0.490
Candida albicans	4	2	0.667
Gardnerella sp.	6	0	0.022
E. coli	4	0	0.110
Micrococcus	1	0	1.000

Table VI:- Intergroup comparison of the levels of micro organisms in the oral cavity of newborn immediately after delivery.

Bacteria	NORMAL DELIVERY	CAESAREAN DELIVERY	p-value (Pearson's Chi-square test)
Sterile	2	3	1.000
Staphylococcus Epidermidis	21	19	0.725
Staphylococcus Aureus	-	-	NA
Streptococcus Salivarius	6	7	1.000
Lactobacillus	9	4	0.196
Klebsiella sp.	3	0	0.235
Neisseria sp.	4	0	0.110
Pseudomonas Aeroginosa	-	-	NA
Candida albicans	1	2	1.000
Gardnerella sp.	-	-	NA
E. coli	1	0	1.000
Micrococcus	-	-	NA

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